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(54) IMPLANTABLE MEDICAL DEVICE WITH A DUAL POWER SOURCE

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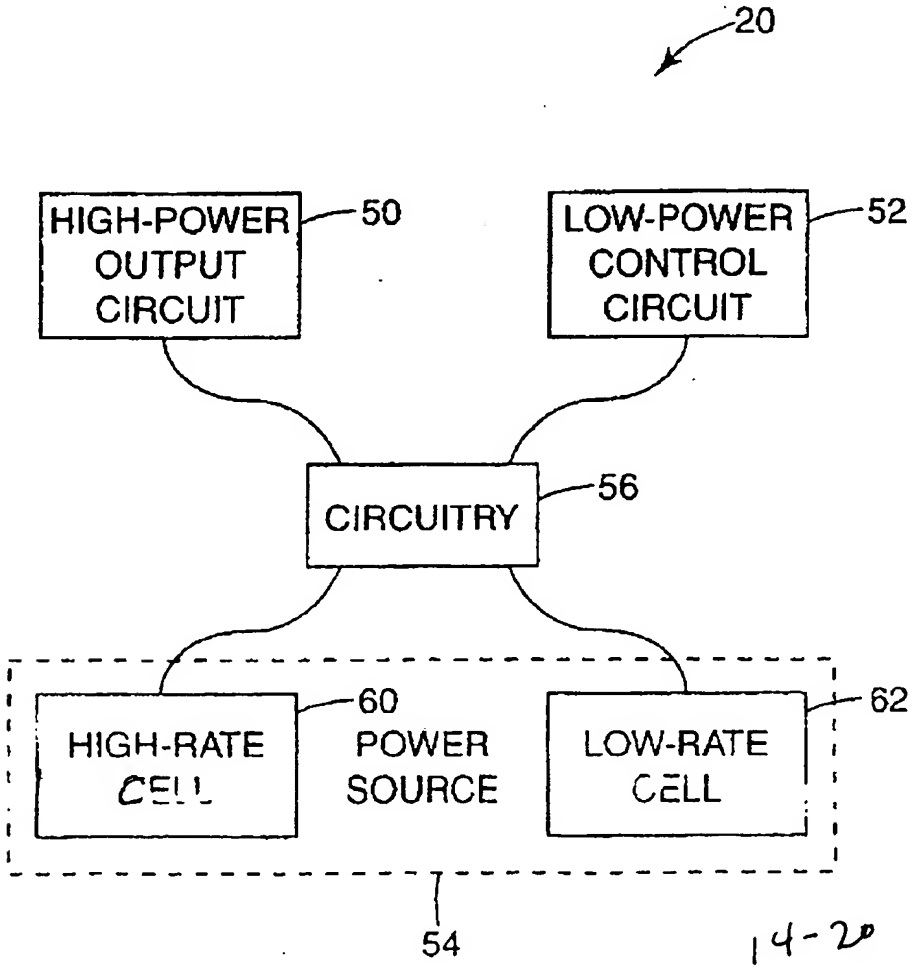
(57) ABSTRACT

An implantable medical device includes a control circuit for controlling the operation of the device and for obtaining physiological data from a patient in which the medical device is implanted. The implanted device also includes a communication circuit for transmitting the physiological data to an external device. A first power source is coupled to the control circuit and provides power to the control circuit. A second power source is coupled to the communication circuit and provides power to the communication circuit.



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0081 claims 4 7

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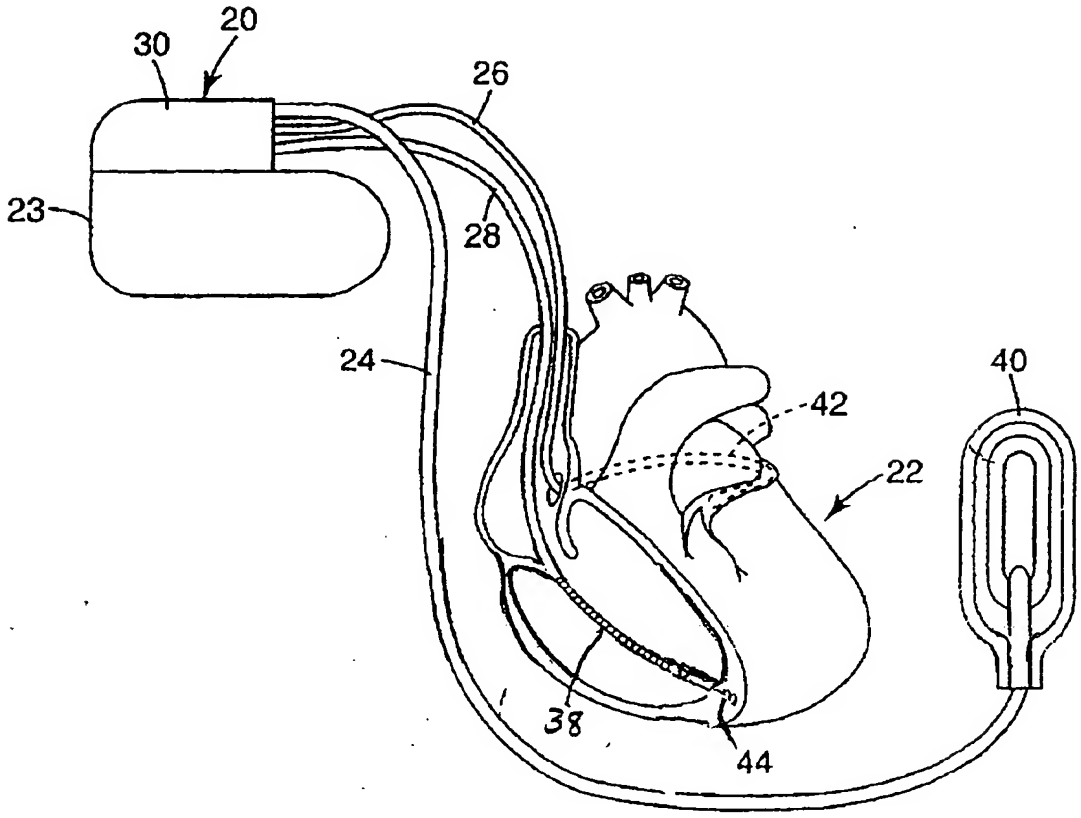

Fig. 1

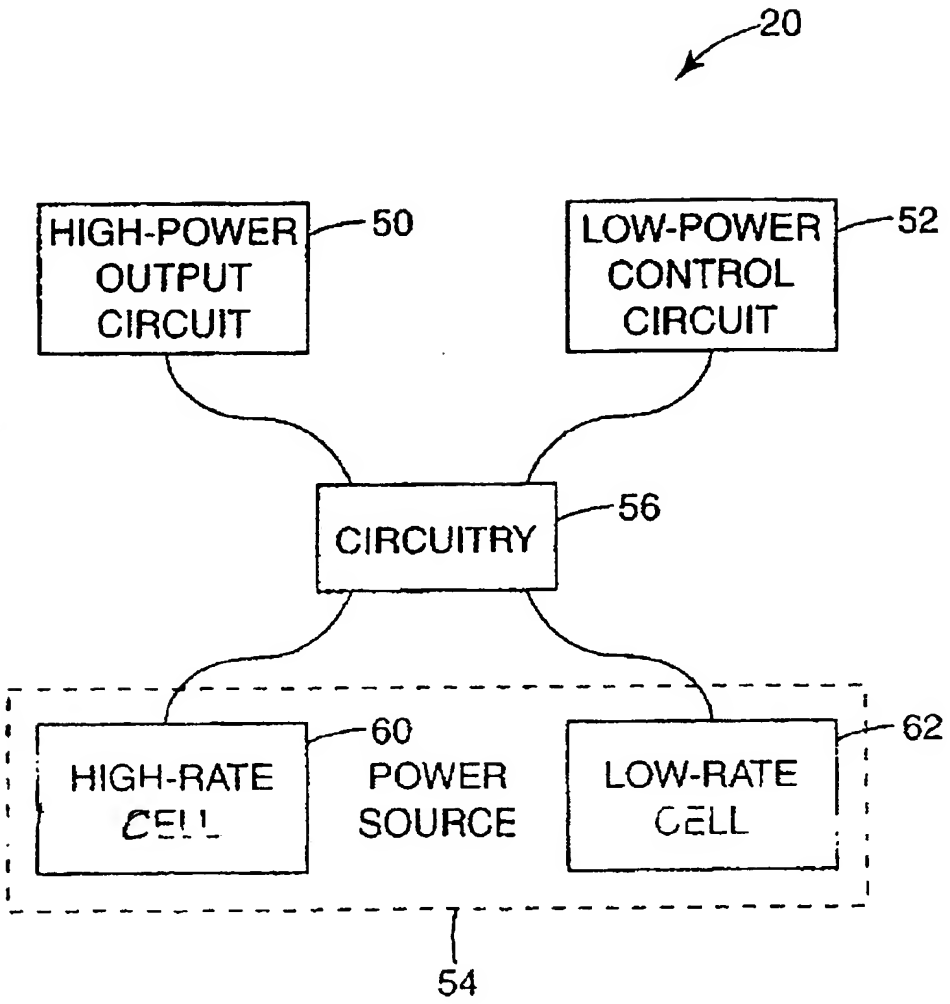
20
HIGH-POWER OUTPUT CIRCUIT
50
LOW-POWER CONTROL CIRCUIT
52
CIRCUITRY
56
HIGH-RATE CELL
60
POWER SOURCE
LOW-RATE CELL
62
54

Fig. 2

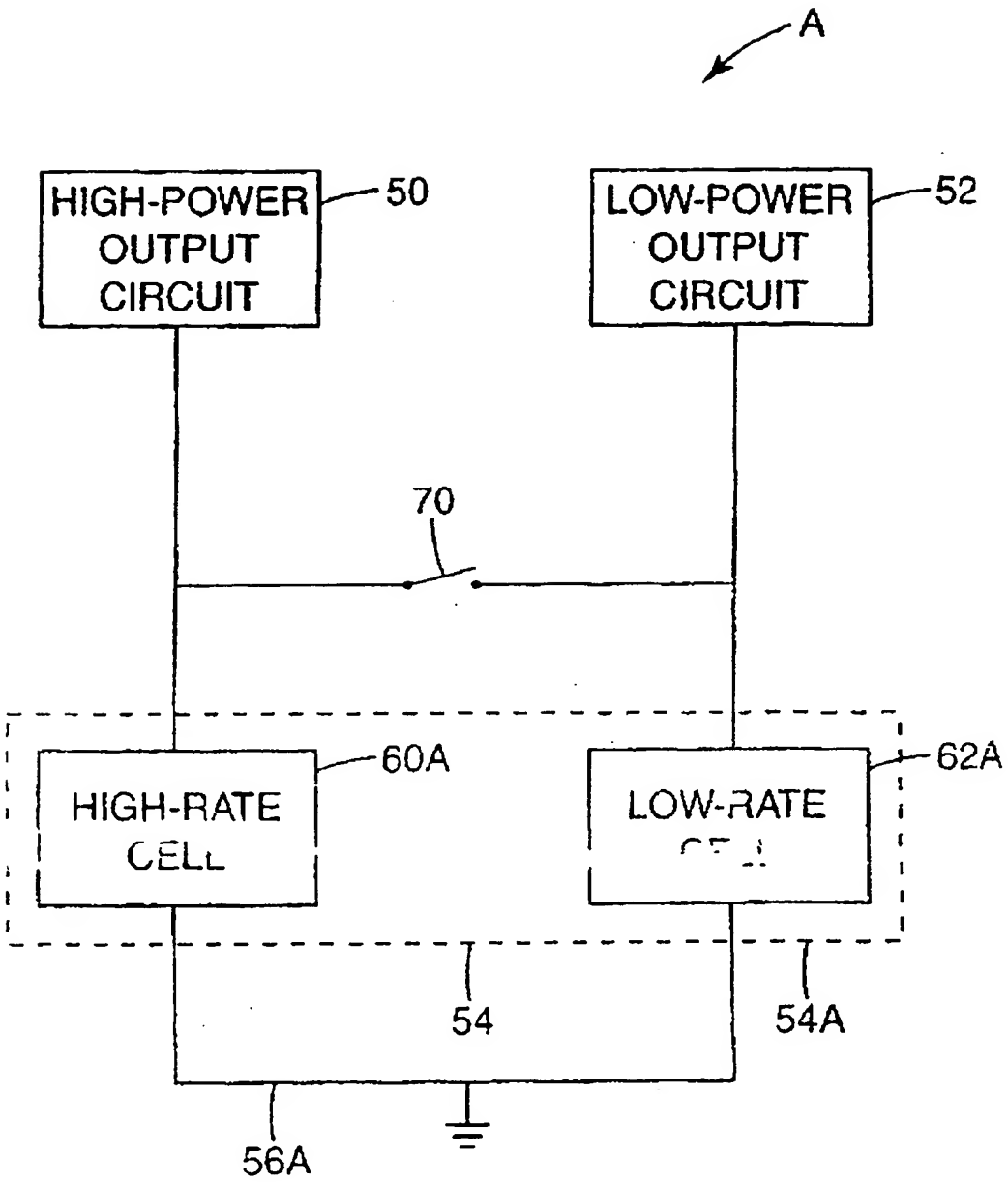
A
HIGH-POWER OUTPUT CIRCUIT
50
LOW-POWER OUTPUT CIRCUIT
52
70
HIGH-RATE CELL
60A
LOW-RATE
62A
54
54A
56A

Fig. 3

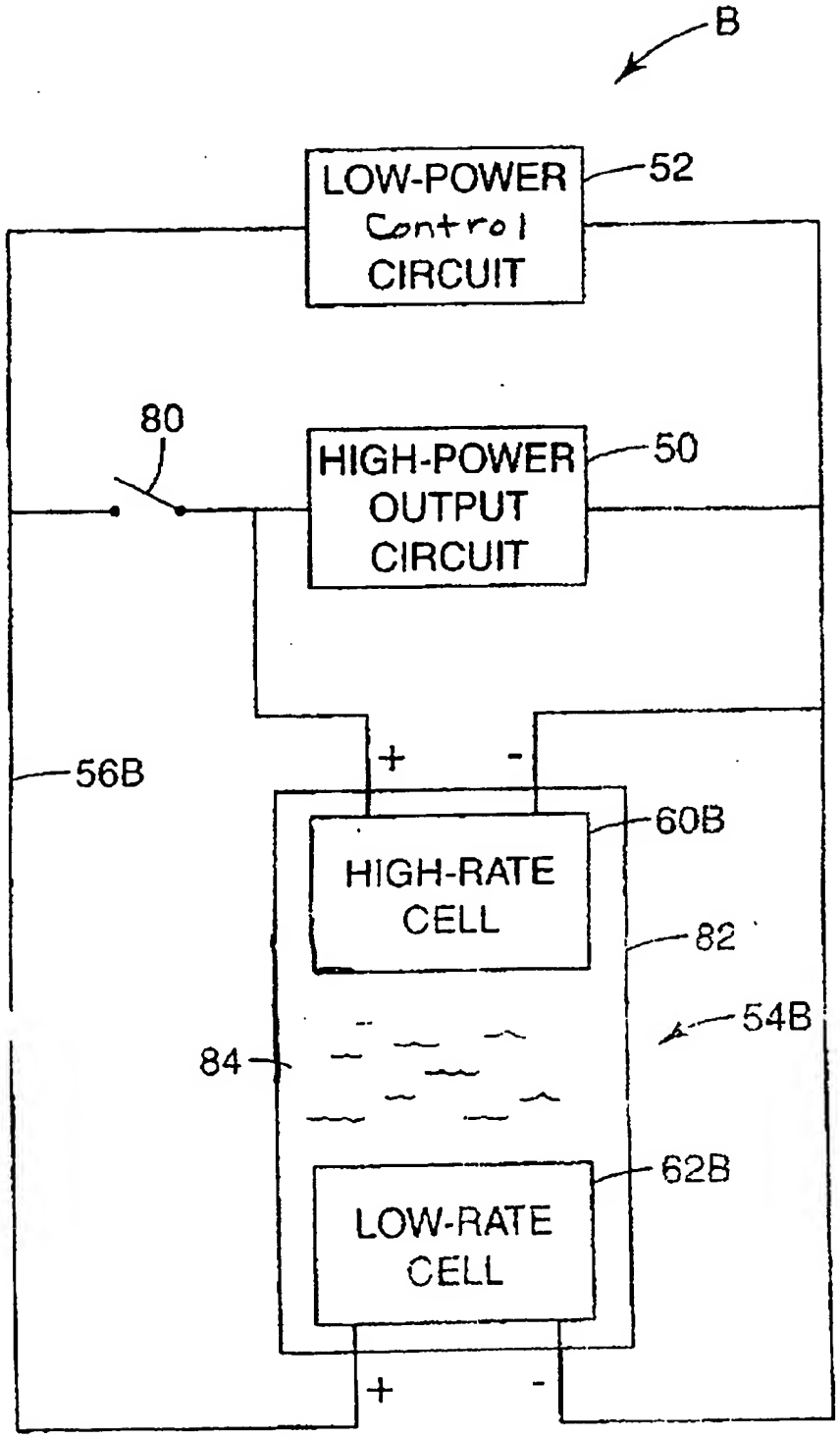
B
LOW-POWER
Control
CIRCUIT
52
80
HIGH-POWER
OUTPUT
CIRCUIT
50
56B
+
-
60B
HIGH-RATE
CELL
82
54B
84
62B
LOW-RATE
CELL
+
-

Fig. 4

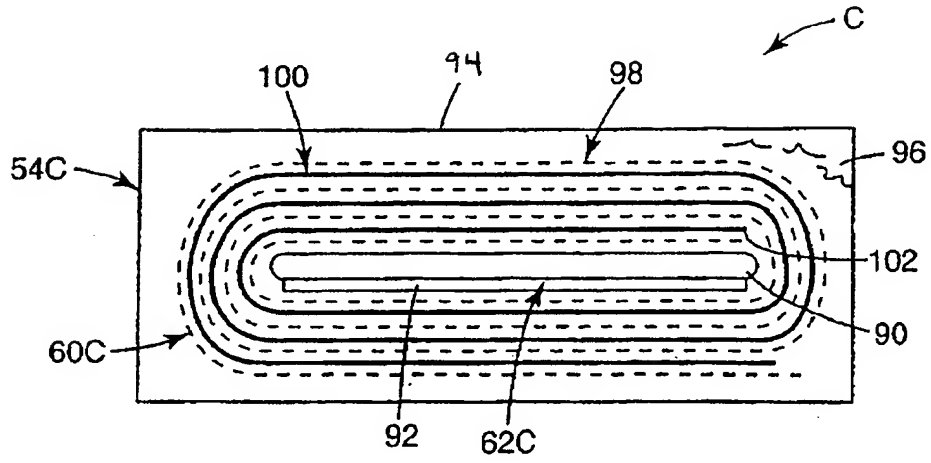

Fig. 5A

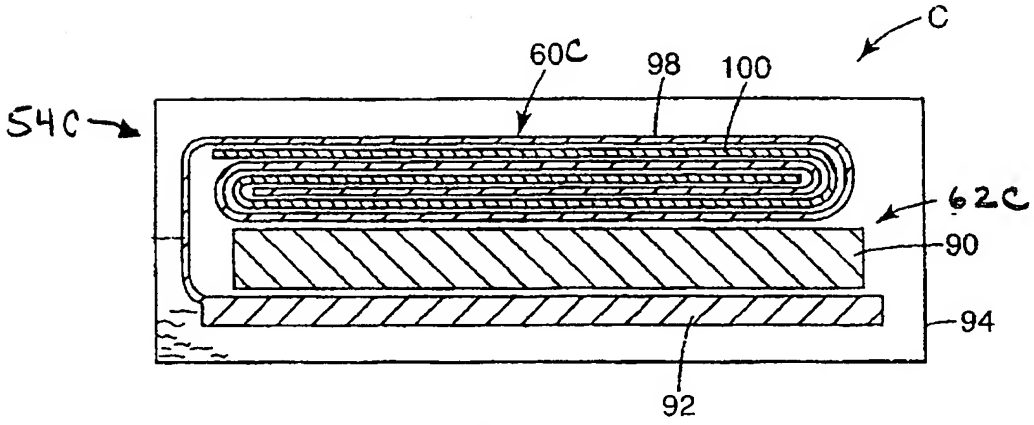

Fig. 5B

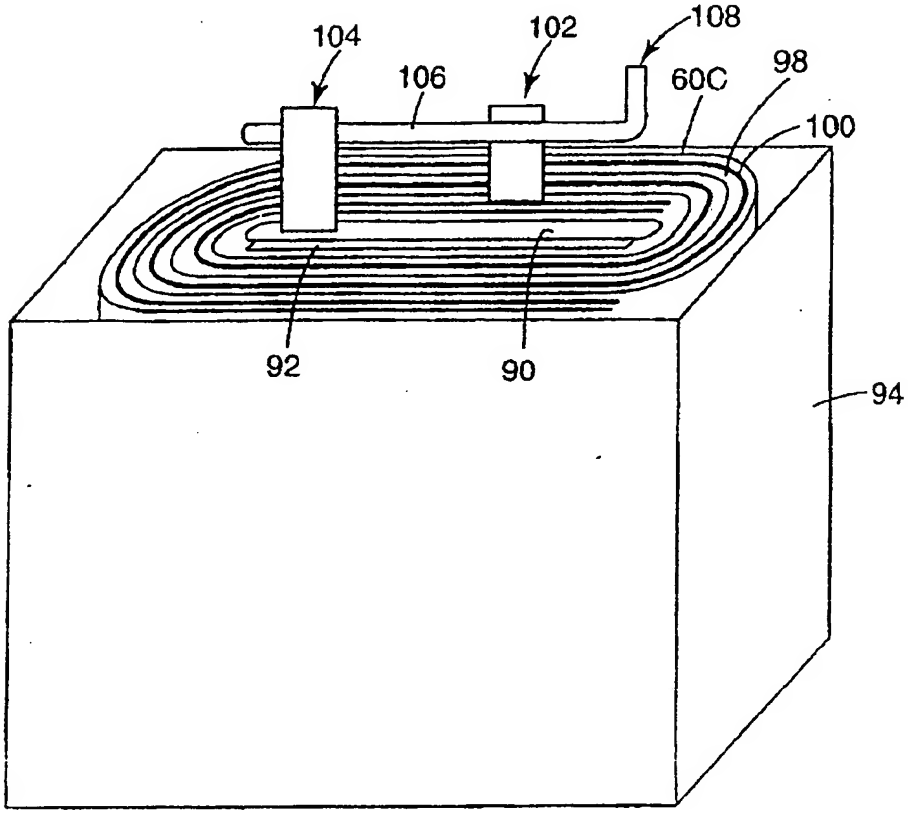

Fig. 5C

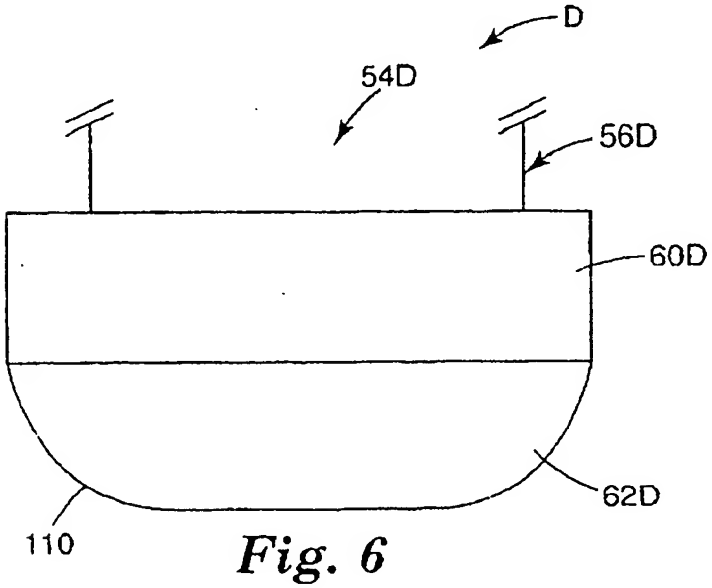

Fig. 6

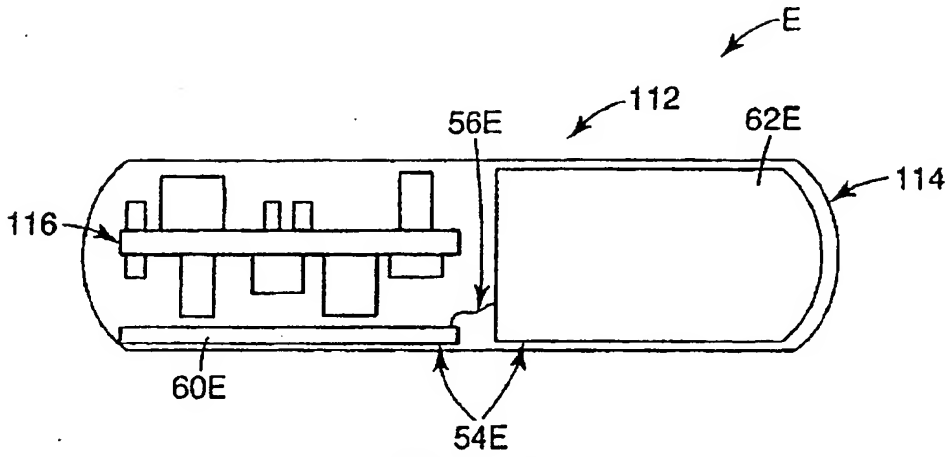
E
112
56E
62E
114
116
60E
54E

Fig. 7

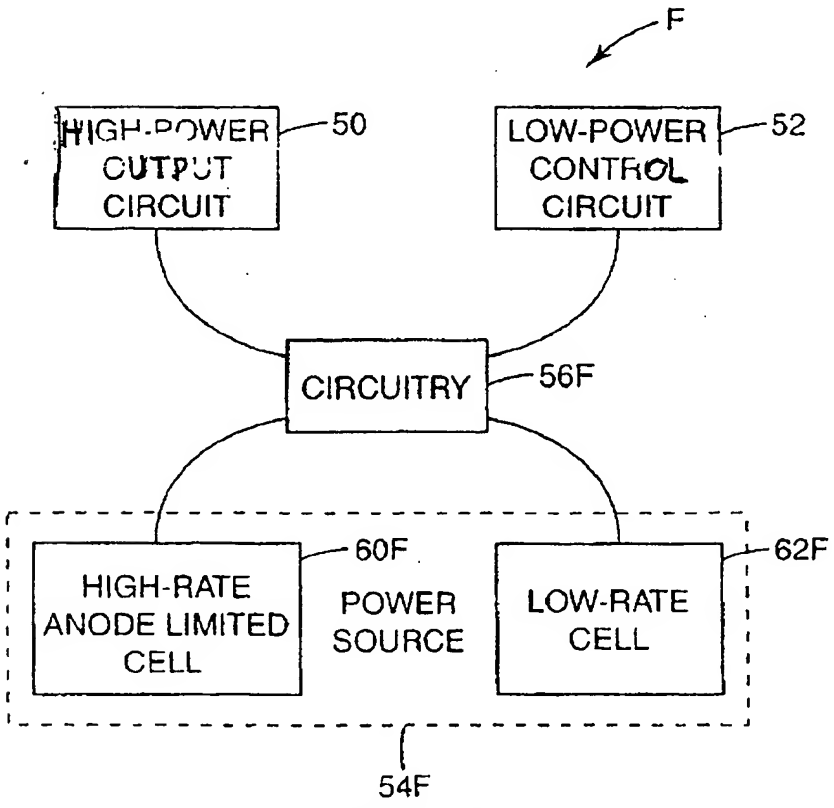
F
HIGH-POWER OUTPUT CIRCUIT
50
LOW-POWER CONTROL CIRCUIT
52
CIRCUITRY
56F
60F
HIGH-RATE ANODE LIMITED CELL
POWER SOURCE
LOW-RATE CELL
62F
54F

Fig. 8

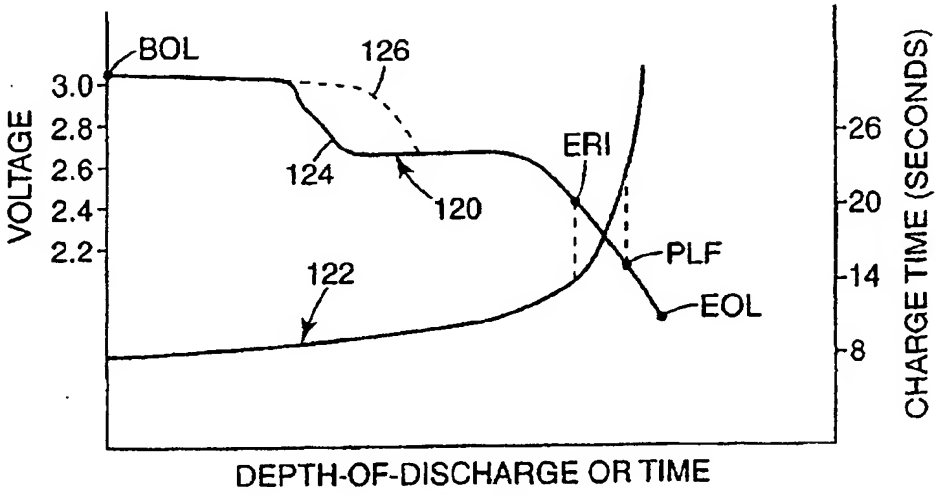
BOL
126
3.0
2.8
2.6
2.4
2.2
VOLTAGE
124
120
ERI
PLF
122
EOL
26
20
14
8
CHARGE TIME (SECONDS)
DEPTH-OF-DISCHARGE OR TIME

Fig. 9

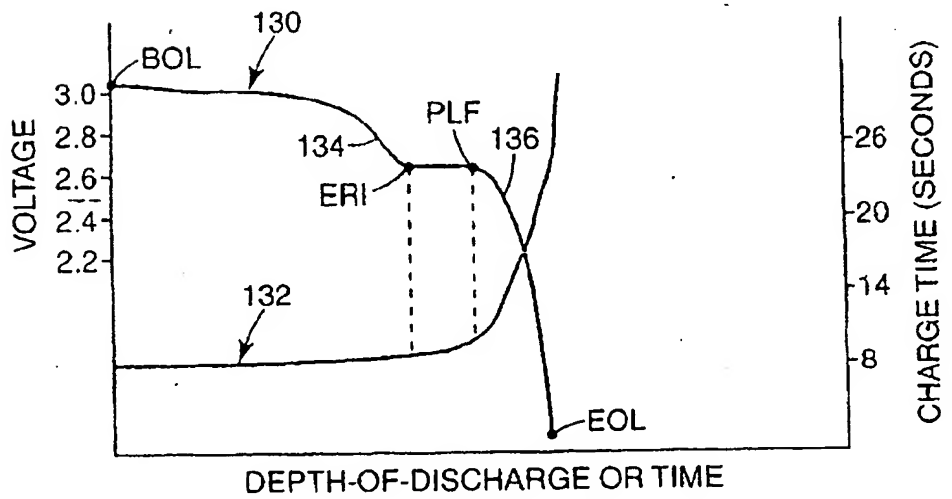
130
BOL
3.0
2.8
2.6
2.4
2.2
VOLTAGE
PLF
134
136
ERI
132
EOL
26
20
14
8
CHARGE TIME (SECONDS)
DEPTH-OF-DISCHARGE OR TIME

Fig. 10

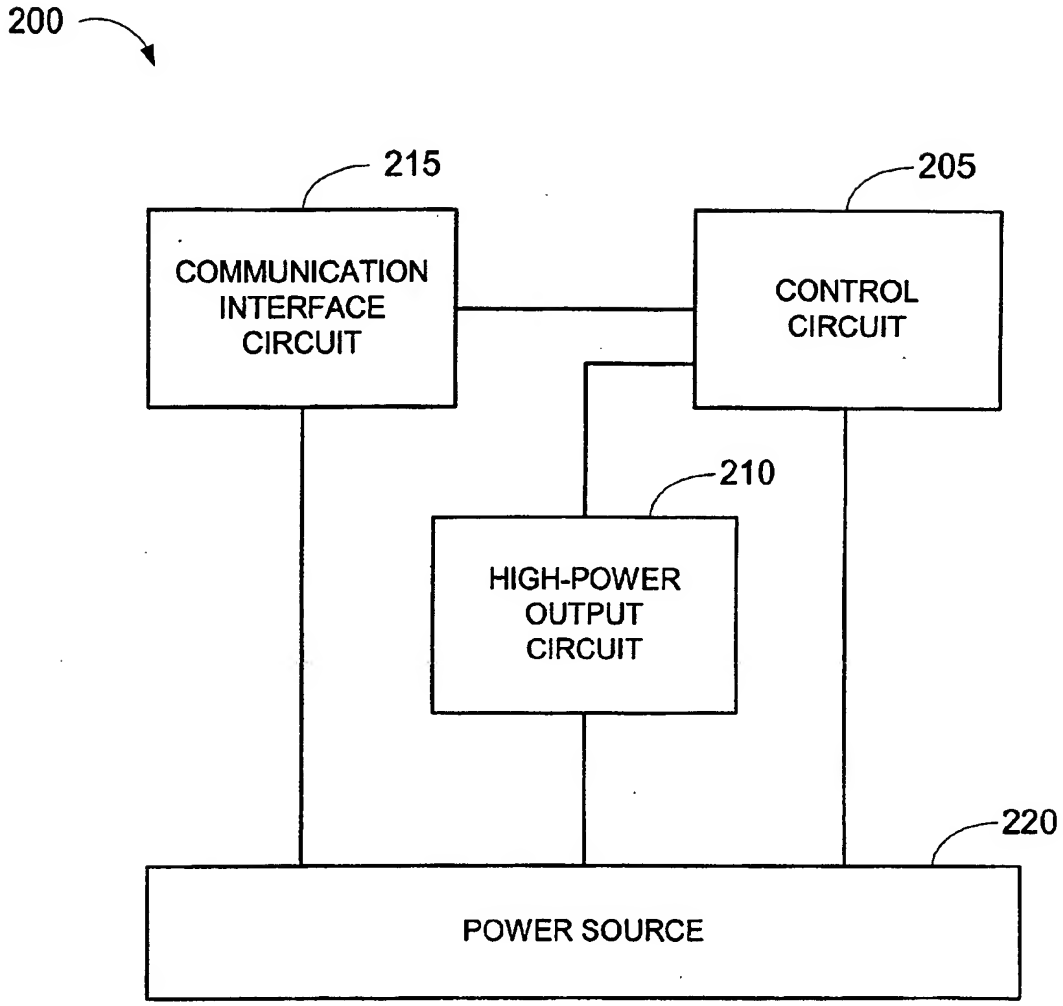
200
215
205
COMMUNICATION INTERFACE CIRCUIT
CONTROL CIRCUIT
210
HIGH-POWER OUTPUT CIRCUIT
220
POWER SOURCE

Fig. 11

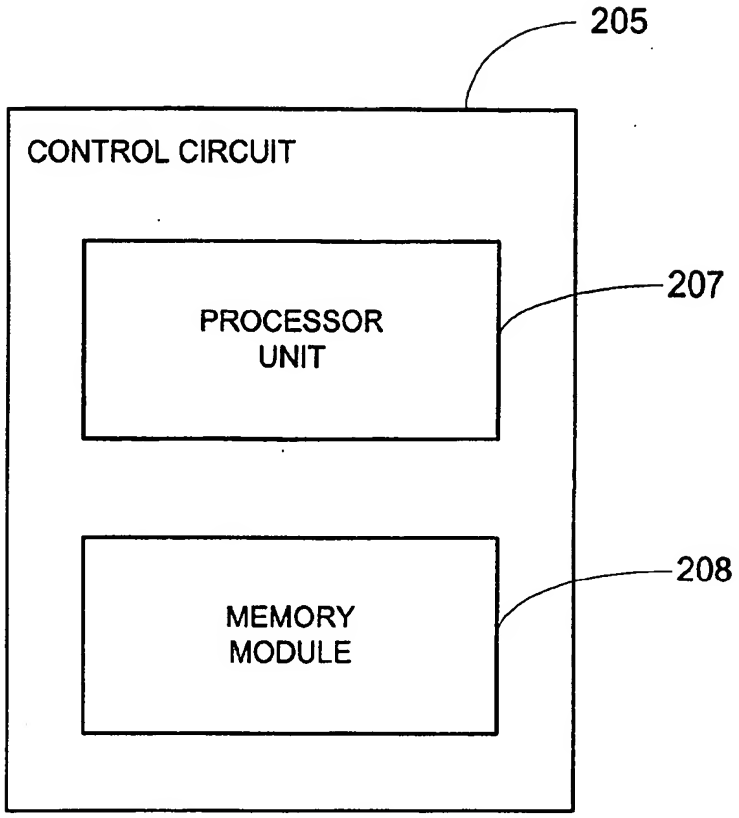

Fig. 11A

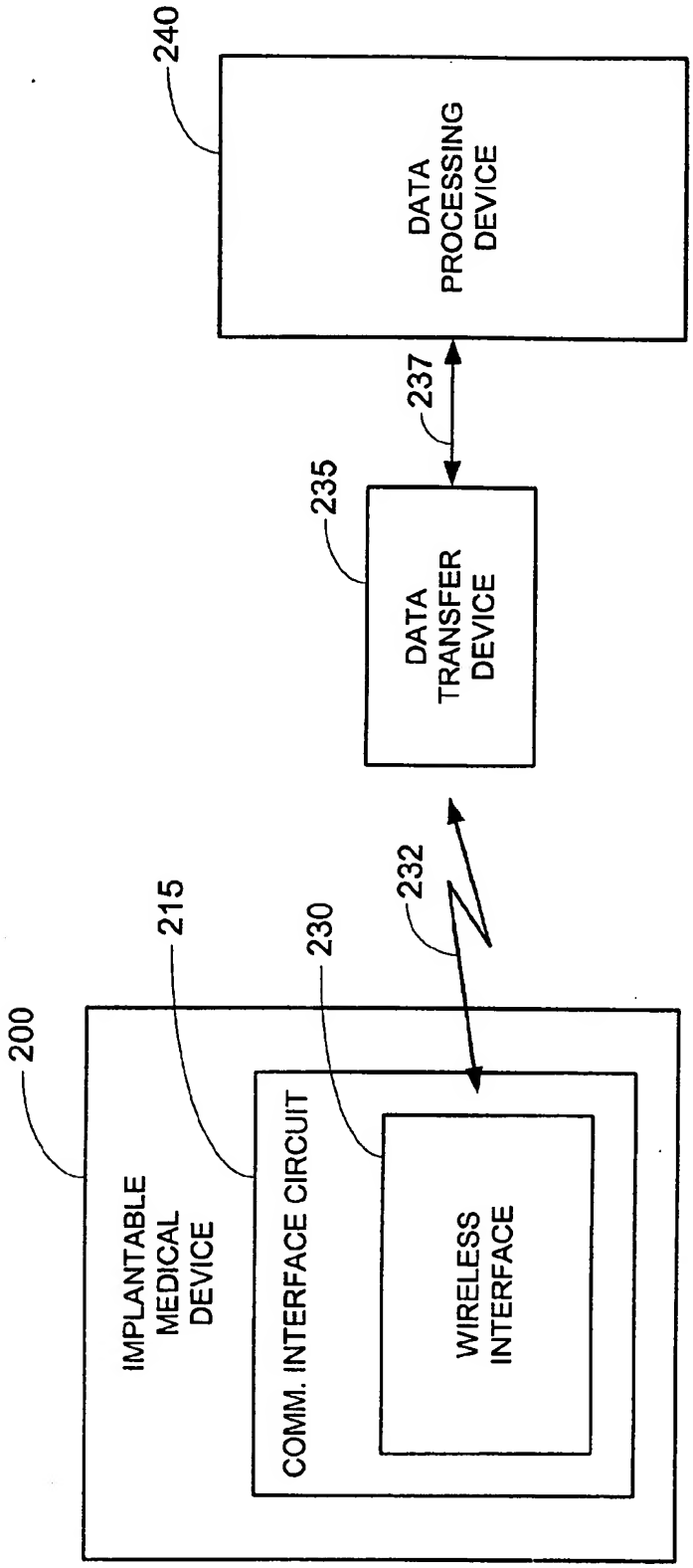
240
DATA PROCESSING DEVICE
237
235
DATA TRANSFER DEVICE
232
215
230
200
IMPLANTABLE MEDICAL DEVICE
COMM. INTERFACE CIRCUIT
WIRELESS INTERFACE

Fig. 12

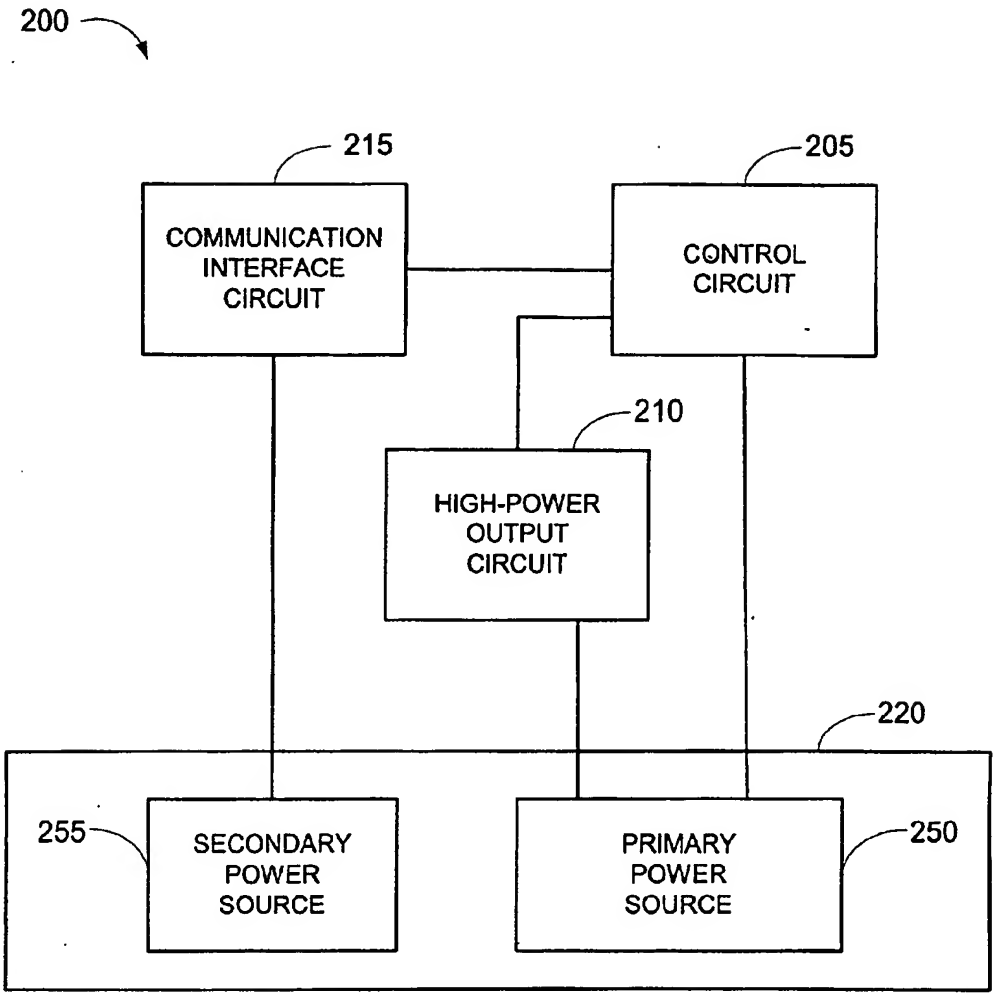
200
215
205
COMMUNICATION INTERFACE CIRCUIT
CONTROL CIRCUIT
210
HIGH-POWER OUTPUT CIRCUIT
220
255
SECONDARY POWER SOURCE
PRIMARY POWER SOURCE
250

Fig. 13

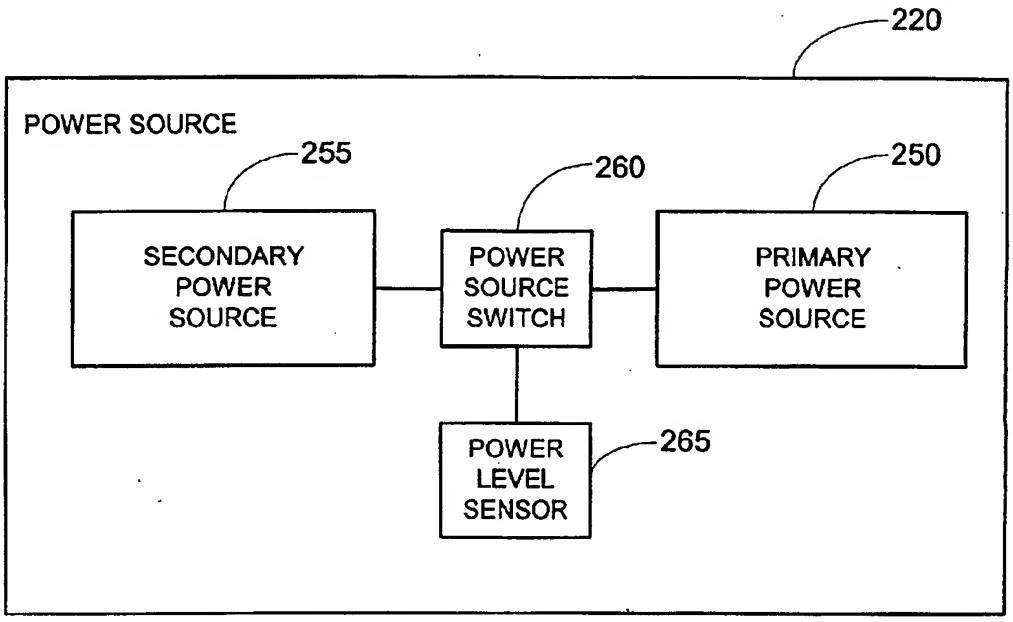
220
POWER SOURCE
255
260
250
SECONDARY POWER SOURCE
POWER SOURCE SWITCH
PRIMARY POWER SOURCE
POWER LEVEL SENSOR
265

Fig. 14

IMPLANTABLE MEDICAL DEVICE WITH A DUAL POWER SOURCE

PRIORITY CLAIM

[0001] This Application is a continuation-in-part of U.S. patent application Ser. No. 09/870,097 (P-7586, filed May 30, 2001, entitled "Implantable Medical Device With a Dual Cell Power Source," which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] The present invention relates generally to a power source for an implantable medical device, and more particularly, the present invention relates to a dual cell power source for optimizing implantable medical device performance.

[0003] A variety of different implantable medical devices (IMD) are available for therapeutic stimulation of the heart and are well known in the art. For example, implantable cardioverter-defibrillators (ICDs) are used to treat those patients suffering from ventricular fibrillation, a chaotic heart rhythm that can quickly result in death if not corrected. In operation, the ICD continuously monitors the electrical activity of a patient's heart, detects ventricular fibrillation, and in response to that detection, delivers appropriate shocks to restore normal heart rhythm. Similarly, an automatic implantable defibrillator (AID) is available for therapeutic stimulation of the heart. In operation, an AID device detects ventricular fibrillation and delivers a non-synchronous high-voltage pulse to the heart through widely spaced electrodes located outside of the heart, thus mimicking transthoratic defibrillation. Yet another example of a prior art cardioverter includes the pacemaker/cardioverter/defibrillator (PCD) disclosed, for example, in U.S. Pat. No. 4,375,817 to Engle, et al. This device detects the onset of tachyarrhythmia and includes means to monitor or detect progression of the tachyarrhythmia so that progressively greater energy levels may be applied to the heart to interrupt a ventricular tachycaria or fibrillation. Numerous other, similar implantable medical devices, for example a programmable pacemaker, are further available.

[0004] Regardless of the exact construction and use, each of the above-described IMDs generally include three primary components: a low-power control circuit, a high-power output circuit, and a power source. The control circuit monitors and determines various operating characteristics, such as, for example, rate, synchronization, pulse width and output voltage of heart stimulating pulses, as well as diagnostic functions such as monitoring the heart. Conversely, the high-power output circuit generates electrical stimulating pulses to be applied to the heart via one or more leads in response to signals from the control circuit.

[0005] The power source provides power to both the low-power control circuit and the high-power output circuit. As a point of reference, the power source is typically required to provide 10-20 microamps to the control circuit and a higher current to the output circuit. Depending upon the particular IMD application, the high-power output circuit may require a stimulation energy of as little as 0.1 Joules for pacemakers to as much as 40 Joules for implantable defibrillators. In addition to providing a sufficient stimulation energy, it is desirable that the power source possess a low self-discharge to have a useful life of many years, and that it is highly reliable, and able to supply energy from a minimum packaged volume.

[0006] Suitable power sources or batteries for IMD's are virtually always electrochemical in nature, commonly referred to as electrochemical cells. Acceptable electrochemical cells for IMDs typically include a case surrounding an anode, a separator, a cathode and an electrolyte. The anode material is typically a lithium metal or, for rechargeable cells, a lithium ion containing body. Lithium batteries are generally regarded as acceptable power sources due in part to their high energy density and low self-discharge characteristics relative to other types of batteries. The cathode material is typically metal-based, such as silver vanadium oxide (SVO), manganese dioxide, etc.

[0007] In some cases, the power requirements of the output circuit are higher than the battery can deliver. Thus, it is common in the prior art to accumulate and store the stimulating pulse energy in an output energy storage device at some point prior to the delivery of a stimulating pulse, such as with an output capacitor. When the control circuit indicates to the output circuit that a stimulating pulse is to be delivered, the output circuitry causes the energy stored in the output capacitor to be applied to the cardiac tissue via the implanted leads. Prior to delivery of a subsequent stimulating pulse, the output capacitor is typically recharged, with the time required for the power source to recharge the output capacitor being referred to as the "charge time".

[0008] Regardless of whether an output capacitor(s) is employed, one perceived drawback of currently known therapeutic pulsing IMDs is that they often have to be replaced before their battery depletion levels have reached a maximum. When an IMD's output capacitor is being recharged, there is a drop in battery voltage due to the charging current flowing through an inherent battery impedance. Although this voltage drop may not be significant when the battery is new or fresh, it may increase substantially as the battery ages or is approaching depletion, such that during a capacitor recharging operation, the voltage supply to the control circuit may drop below a minimum allowable level. This temporary drop can cause the control circuit to malfunction. The IMD may be removed and replaced before any such malfunctions occur, even though the battery may still have sufficient capacity to stimulate the heart. Simply stated, the rate capability of currently available lithium-based cells is highly dependent upon time or depth-of-discharge as the cell develops high internal resistance over time and/or with repeated use. For IMD applications, this time or depth-of-discharge dependence limits the battery's useful life.

[0009] One solution to the above-described issue is to provide two batteries, one for charging the output circuit or capacitor and a separate battery for powering the control circuit. Unfortunately, the relative amounts of energy required by the device for the control and charging/output circuitry tend to vary from patient to patient. The capacity of the battery to power the control circuit can only be optimized with regard to one patient profile. Thus for other patients, one battery may deplete before the other, leaving wasted energy in the device. An example of such a system is disclosed in U.S. Pat. No. 5,614,331 to Takeuchi et al.

[0010] An additional, related concern associated with IMD power sources relates to overall size constraints. In

particular, in order to provide an appropriate power level for a relatively long time period (on the order of 4-7 years), the power source associated with the high-power output circuitry typically has a certain electrode surface area to achieve the high-rate capability. Due to safety and fabrication constraints, the requisite electrode surface area can be achieved with an increased cell volume. The resulting cell may satisfy output circuitry power requirements, but unfortunately may be volumetrically inefficient. Even further, recent IMD designs require the power source to assume a shape other than rectangular, such as a "D" or half "D" contour, further contributing to volumetric inefficiencies.

[0011] In general terms, then, currently available electrochemical cell designs, especially Li/SVO constructions, may satisfy, at least initially, power requirements for the output circuitry. The inherent volumetric inefficiencies of these cells, however, dictates an end-of-life point at which less than the cell's useful capacity has been used. Once again, currently available cells exhibit an output circuitry charge time that is highly dependent upon time of use or depth-of-discharge. Over time, the cell's impedance increases, thereby increasing the resulting charge time. Virtually all IMDs have a maximum allowable charge time for the output circuitry. Once the cell's charge time exceeds the maximum allowable charge time, the IMD may be replaced. The volumetrically inefficient cell may quickly reach this maximum charge time, even though a large portion of the cell's capacity remains unused (on the order of 40% of the useful capacity). Thus, regardless of whether the power source incorporates one or two cells, the resulting configuration is highly inefficient in terms of the high-rate battery's useful capacity.

[0012] Manufacturers continue to improve upon IMD construction and size characteristics. To this end, currently available power source designs are less than optimal. Therefore, a need exists for an IMD power source having superior space-volumetric efficiencies and a higher energy density, without a proportional increase in charge time.

[0013] Yet another issue associated with IMD power sources involves the use of a wireless transceiver to communicate IMD data with an external device. The data communicated by the IMD may include physiological data related to the patient in which the IMD is implanted. For example, if the IMD is a pacemaker or cardioverter/defibrillator, the physiological data may include electric cardiac signals obtained from electrodes implanted within the patient's heart as previously discussed. The external device with which the IMD communicates this physiological data may include a computer, for example, that monitors and/or processes the physiological data that is received from the IMD.

[0014] The IMD may also communicate data related to its performance, such as the intensity level in which it delivered a therapeutic shock for a given set of electric cardiac signals monitored via the implanted electrodes. The external computer device may analyze the received data and transmit programming data to the IMD to adjust its therapy. For example, the programming data may indicate to the IMD to reduce the intensity of the therapeutic shock delivered to the patient.

[0015] Typically, the wireless transceiver within the IMD requires relatively high current pulses, thus resulting in a higher drain from the power source within the IMD. As the sophistication of the IMD and the number of communication transmissions performed by the IMD is expected to increase over the next several years, a much higher burden may be placed on the IMD's power source, thus reducing its life. Because the accessibility of the power source is achieved typically via a surgical procedure, this reduction in battery life is a concern.

[0016] The present invention is directed to reducing the effects of one or more of the problems set forth above.

SUMMARY OF THE INVENTION

[0017] According to the present invention, an apparatus includes a control circuit coupled to a first power source to control the operation of the apparatus, the control circuit being adapted to receive power from the first power source. A communication circuit is coupled to a second power supply to communicate with an external device, the communication circuit being adapted to receive power from the second power source.

[0018] According to the present invention, an implantable medical device includes a control circuit to control the operation of implantable the medical device and to obtain physiological data from a patient in which the implantable medical device is implanted. A communication circuit is coupled to the control circuit to transmit the physiological data to an external device, a first power source is coupled to the control circuit to provide power to the control circuit, and a second power source is coupled to the communication circuit to provide power to the communication circuit.

[0019] According to the present invention, a method for incorporating a power source in an implantable medical device includes providing power to a control circuit by a first power source, the control circuit obtaining physiological data of a patient in which at least the control circuit is implanted; providing power to a communication circuit by a second power source; and transmitting the physiological data from the communication circuit to an external device.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] **FIG. 1** is a simplified schematic view of one embodiment of an implantable medical device (IMD) incorporating a power source in accordance with the present invention;

[0021] **FIG. 2** is a simplified schematic circuit diagram of a power source in accordance with the present invention for use with the IMD of **FIG. 1**;

[0022] **FIG. 3** is a simplified schematic diagram of a first embodiment power source in accordance with the present invention;

[0023] **FIG. 4** is a simplified schematic diagram of a second embodiment power source in accordance with the present invention;

[0024] **FIG. 5A** is a cross-sectional view of a third alternative embodiment power source in accordance with the present invention;

[0025] **FIG. 5B** is a cross-sectional view of a variation of the embodiment of **FIG. 5A**;

[0026] FIG. 5C is a perspective view of the power source of FIG. 5A including an internal, parallel connection;

[0027] FIG. 6 is a top view of a fourth embodiment power source in accordance with the present invention;

[0028] FIG. 7 is a cross-sectional view of an IMD incorporating a fifth embodiment power source;

[0029] FIG. 8 is a simplified schematic diagram of a sixth embodiment power source;

[0030] FIG. 9 is a graph showing a discharge curve for a conventionally balanced battery;

[0031] FIG. 10 is a graph showing a discharge curve for an anode limited battery for use with the power source of FIG. 8;

[0032] FIG. 11 is a simplified block diagram of an implantable medical device (IMD) incorporating a power source in accordance with another embodiment of the present invention;

[0033] FIG. 11A is a more detailed representation of a control circuit of the IMD of FIG. 11;

[0034] FIG. 12 illustrates the communication capabilities of the IMD of FIG. 11 with an external data processing device;

[0035] FIG. 13 is a more detailed representation of the power source of the IMD of FIG. 11 in accordance with one embodiment of the present invention; and

[0036] FIG. 14 illustrates another more detailed representation of the power source of the IMD of FIG. 13 according to another embodiment of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0037] FIG. 1 is a simplified schematic view of one embodiment of an implantable medical device ("IMD") 20 in accordance with the present invention and its relationship to a human heart 22. The IMD 20 is shown in FIG. 1 as preferably being a pacemaker/cardioverter/defibrillator (PCD), although the IMD may alternatively be a drug delivery device, a neurostimulator, or any other type of implantable device known in the art. The IMD includes a case or hermetic enclosure 23 and associated electrical leads 24, 26 and 28. As described in greater detail below, the enclosure case 23 contains various circuits and a power source. The leads 24, 26 and 28 are coupled to the IMD 20 by means of a multi-port connector block 30, which contains separate ports for each of the three leads 24, 26, and 28 illustrated.

[0038] In one embodiment, lead 24 is coupled to a subcutaneous electrode 40, which is intended to be mounted subcutaneously in the region of the left chest. Alternatively, an active "can" may be employed such that stimulation is provided between an implanted electrode and enclosure case 23. In yet another embodiment, stimulation is provided between two electrodes carried on a single multipolar lead.

[0039] The lead 26 is a coronary sinus lead employing an elongated coil electrode that is located in the coronary sinus and great vein region of the heart 22. The location of the electrode is illustrated in broken line format at 42, and extends around the heart 22 from a point within the opening of the coronary sinus to a point in the vicinity of the left atrial appendage.

[0040] Lead 28 is provided with an elongated electrode coil 38 which is located in the right ventricle of the heart 22. The lead 28 also includes a helical stimulation electrode 44 which takes the form of an extendable/retractable helical coil which is screwed into the myocardial tissue of the right ventricle. The lead 28 may also include one or more additional electrodes for near and far field electrogram sensing.

[0041] In the system illustrated, cardiac pacing pulses are delivered between the helical electrode 44 and the coil electrode 38. The electrodes 38 and 44 are also employed to sense electrical signals indicative of ventricular contractions. Additionally, cardioverters/defibrillation shocks may be delivered between coil electrode 38 and the electrode 40, and between coil electrode 38 and electrode 42. During sequential pulse defibrillation, it is envisioned that pulses would be delivered sequentially between subcutaneous electrode 40 and coil electrode 38, and between the coronary sinus electrode 42 and coil electrode 38. Single pulse, two electrode defibrillation pulse regimens may also be provided, typically between coil electrode 38 and the coronary sinus electrode 42. Alternatively, single pulses may be delivered between electrodes 38 and 40. The particular interconnection of the electrodes to the IMD 20 will depend somewhat on the specific single electrode pair defibrillation pulse regimen is believed more likely to be employed.

[0042] Regardless of the exact configuration and operation of the IMD 20, the IMD 20 includes several basic components, illustrated in block form in FIG. 2. The IMD 20 includes a high-power output circuit 50, a low-power control circuit 52, a power source 54 (shown with dashed lines) and circuitry 56. As described in greater detail below, the power source 54 is preferably a dual-cell configuration, and can assume a wide variety of forms. Similarly, the circuitry 56 can include analog and/or digital circuits, can assume a variety of configurations, and electrically connects the power source 54 to the high power circuit 50 and the low-power circuit 52.

[0043] The high-power output circuit 50 and the low-power control circuit 52 are typically provided as part of an electronics module associated with the IMD 20. In general terms, the high-power output circuit 50 is configured to deliver an electrical pulse therapy, such as a defibrillation or a cardioversion/defibrillation pulse. In sum, the high-power output circuit 50 is responsible for applying stimulating pulse energy between the various electrodes 38-44 (FIG. 1) of the IMD 20. As is known in the art, the high-power output circuit 50 may be associated with a capacitor bank (not shown) for generating an appropriate output energy, for example in the range of 0.1-40 Joules.

[0044] The low-power control circuit 52 is similarly well known in the art. In general terms, the low-power control circuit 52 monitors heart activity and signals activation of the high-power output circuit 50 for delivery of an appropriate stimulation therapy. Further, as known in the art, the low-power control circuit 52 may generate a preferred series of pulses from the high-power output circuit 50 as part of an overall therapy.

[0045] The power source 54 and associated circuitry 56 can assume a wide variety of configurations, as described in

the various embodiments below. Preferably, however, the power source **54** includes a first, high-rate cell **60** and a second, lower-rate cell **62**, such as a medium- or low-rate cell. Notably the first and second cells **60, 62** can be formed separate from one another or contained within a singular enclosure. Depending upon the particular application, the high-rate cell **60** is configured to provide a stimulation energy of as little as 0.1 Joules for pacemakers to as much as 40 Joules for implantable defibrillators. As described below with reference to specific embodiments, the high-rate cell **60** can assume a wide variety of forms as is known in the art. Preferably, the high-rate cell **60** includes an anode, a cathode and an electrolyte. The anode is preferably formed to include lithium, either in metallic form or ion form for re-chargeable applications. With this in mind, the high-rate cell **60** is most preferably a spirally-wound battery of the type disclosed, for example, in U.S. Pat. No. 5,439,760 to Howard et al. for "High Reliability Electrochemical Cell and Electrode Assembly Therefor" and U.S. Pat. No. 5,434,017 to Berkowitz et al. for "High Reliability Electrochemical Cell and Assembly Therefor," the disclosures of which are hereby incorporated by reference. The high-rate cell **60** may less preferably be a battery having a spirally-wound, stacked plate or serpentine electrodes of the type disclosed, for example, in U.S. Pat. Nos. 5,312,458 and 5,250,373 to Muffuletto et al. for "Internal Electrode and Assembly Method for Electrochemical Cells;" U.S. Pat. No. 5,549,717 to Takeuchi et al. for "Method of Making Prismatic Cell;" U.S. Pat. No. 4,964,877 to Kiester et al. for "Non-aqueous Lithium Battery;" U.S. Pat. No. 5,14,737 to Post et al. for "Electrochemical Cell With Improved Efficiency Serpentine Electrode;" and U.S. Pat. No. 5,468,569 to Pyszczek et al. for "Use of Standard Uniform Electrode Components in Cells of Either High or Low Surface Area Design," the disclosures of which are herein incorporated by reference. Alternatively, the high-rate cell **60** can include a single cathode electrode.

[0046] Materials for the cathode of the high-rate cell **60** are most preferably solid and comprise as active components thereof metal oxides such as vanadium oxide, silver vanadium oxide (SVO) or manganese dioxide, as is known in the art. Alternatively, the cathode for the high-rate cell **60** may also comprise carbon monofluoride and hybrids thereof or any other active electrolytic components and combination. Where SVO is employed for the cathode, the SVO is most preferably of the type known as "combination silver vanadium oxide" (or "CSVO") as disclosed in U.S. Pat. Nos. 5,221,453; 5,439,760; and 5,306,581 to Crespi et al, although other types of SVO may be employed.

[0047] It is to be understood that electrochemical systems other than those set forth explicitly above may also be utilized for the high-rate cell **60**, including, but not limited to, anode/cathode systems such as lithium/silver oxide; lithium/manganese oxide; lithium/V_2O_5; lithium/copper silver vanadium oxide; lithium/copper oxide; lithium/lead oxide; lithium/carbon monofluoride; lithium/chromium oxide; lithium/bismuth-containing oxide; lithium/copper sulfate; mixtures of various cathode materials listed above such as a mixture of silver vanadium oxide and carbon monofluoride; and lithium ion rechargeable batteries, to name but a few.

[0048] In general terms, the second, lower-rate cell **62** has a rate capability that is less than that of the high-rate cell **60**, and is sufficient to power the low-power control circuit **52**. For example, in one preferred embodiment, the second, lower-rate cell **62** is a medium rate, SVO cell, more preferably SVO/CF_x cell. Alternatively, the second, lower-rate cell **62** can be a low-rate, lithium/iodine pacemaker battery having a current drain in the range of 10-30 microamps. As known in the art, acceptable constructions of the second, lower-rate cell **62** include, for example, a single cathode electrode design described in U.S. Pat. No. 5,716,729 to Sunderland et al. for "Electrochemical Cell," the disclosure of which is incorporated by reference. As used throughout the specification, reference to a "lower-rate cell" includes both a low-rate cell and a medium-rate cell. Regardless of the exact construction, the high rate cell **60** and the lower-rate cell **62** preferably have similar beginning of life (BOL) voltages (e.g., less than 100 mV). Further, it is preferred that the cells **60, 62** have similar depletion voltages so that the capacity of each of the cells **60, 62** is efficiently used when the first of the cells **60** or **62** reaches depletion.

[0049] With the above-described parameters of the high-rate cell **60** and the second, lower-rate cell **62** in mind, one preferred combination A of a power source **54A** and circuitry **56A** is depicted schematically in **FIG. 3**. The power source **54A** includes a first, high-rate cell **60A** and a second, lower-rate cell **62A** as described above. In addition, circuitry **56A** electrically connects the high-rate cell **60A** and the lower-rate cell **62A** in parallel to the high-power output circuit **50** and the low-power control circuit **52**. In particular, the circuitry **56A** includes a switch **70** configured to selectively uncouple the high-rate cell **60** from the low-power control circuit **52**. In this regard, the circuitry **56A** can include additional components/connections (not shown) for activating and deactivating the switch **70** in response to operational conditions described below.

[0050] The power source/circuitry configuration A provides a distinct advantage over prior art, single-cell designs. For example, during operation of the IMD **20** (**FIG. 1**), the power source **54A** is, from time-to-time, required to deliver a high-current pulse or charge to the high-power output circuit **50** while maintaining a voltage high enough to continuously power the low-power control circuit **52**. If the supply voltage drops below a certain value, the IMD **20** will cease operation. The power source/circuitry configuration A places the high-rate cell **60A** and the lower-rate cell **62A** in parallel to power the low-power control circuit **52** during periods when the high-power output circuit **50** is not activated. During a transient high power pulse, such as a defibrillation pulse, the switch **70** is opened to uncouple the high-rate cell **60A** from the low-power control circuit **52**. The lower-rate cell **62A** remains electrically connected to the low-power control circuit **52**. Thus, the lower-rate cell **62A** continuously powers the low-power control circuit **52**, regardless of any voltage drop experienced by the high-rate cell **60A**. With the parallel configuration of the circuitry **56A**, the high-rate cell **60A** and the lower-rate cell **62A** can be operated in combination for approximately the entire useful life of the respective cells **60A, 62A**. Further, where desired, the cells **60A** and/or **62A** can be sized and shaped to satisfy certain volumetric or shape constraints presented by the IMD **20** (**FIG. 1**).

[0051] An alternative embodiment power source/circuitry configuration B is depicted schematically in **FIG. 4**. The power source/circuitry configuration B includes a power

source **54B** and circuitry **56B**. The power source **54B** includes a first, high-rate cell **60B** and a second, lower-rate cell **62B**. The circuitry **56B** connects the high-rate cell **60B** and the lower-rate cell **62B** in parallel with the high-power output circuit **50** and the low-power control circuit **52**, and includes a switch **80**. The switch **80** is configured to selectively uncouple the high-rate cell **60B** from the low-power control circuit **52**, such that the circuitry **56B** can include additional components/connections (not shown) for activating and deactivating the switch **80** in response to operational conditions described below.

[0052] The power source **54B** is preferably a reservoir battery whereby both the high-rate cell **60B** and the lower-rate cell **62B** are maintained within a single case, shown generally at **82**. In this regard, the high-rate cell **60B** includes an anode/cathode combination that is electrochemically correlated (preferably identical) with an anode/cathode construction of the lower-rate cell **62B** such that a common electrolyte **84** activates both cells **60B**, **62B**. For example, the high-rate cell **60B** can be a high-rate Li/SVO, whereas the lower-rate cell **62B** is a high-volumetric efficiency cell such as Li/SVO or a Li/MnO_2 cell with a pellet design. Alternatively, other constructions for the cells **60B**, **62B**, as previously described, are equally acceptable.

[0053] Connecting the cells **60B**, **62B** in parallel, via the circuitry **56B**, to the high-power output circuit **50** and the low-power control circuit **52** allows for both cells **60B**, **62B** to power the low-power control circuit **52**, thereby extending the useful life of the power source **54B**. Further, as with the power source/circuitry configuration A (**FIG. 3**) previously described, the switch **80** ensures low-power control circuit **52** operation during transient high power pulses by the high-power output circuit **50**. For example, when the high power output circuit **50** is prompted to deliver a high power pulse or charge, the circuitry **56B** opens the switch **80** to uncouple the high-rate cell **60B** from the low-power control circuit **52**. The lower-rate cell **62B** remains electrically connected, providing continuous, uninterrupted power to the low-power control circuit **52**.

[0054] In addition, the lower-rate cell **62B** can serve to recharge the high-rate cell **60B**. More particularly, after the high-rate cell **60B** is pulsed, the potential of the high-rate cell **60B** will be lower than that of the lower-rate cell **62B**. When the lower-rate cell **62B** is re-connected to the high-rate cell **60B** (via the switch **80**), the lower-rate cell **62B** will be discharged and the high-rate cell **60B** correspondingly charged until they reach equal potentials. Electrons move from the anode of the lower-rate cell **62B** to the anode of the high-rate cell **60B**, and from the cathode of the high-rate cell **60B** to the cathode of the lower-rate cell **62B**. In one preferred embodiment, for recharging to occur, the high-rate cell **60B** must possess at least some degree of rechargeability. That is to say, the high-rate cell **60B** may not be rechargeable per the above description if discharged to a high degree. It has been found that configuring the high-rate cell **60B** to exhibit a "micro-rechargeability" characteristic allows the small amount of capacity removed during operation of the high-power output circuit **50** (e.g., a therapy) to be replaced. It has further been found that a high-rate cell **60B** including an SVO cathode exhibits this desired micro-rechargeability characteristic. Alternatively, other cathode materials may also be acceptable. Notably, this same recharging mechanism applies to the configuration A (**FIG. 3**) previously described.

[0055] As an additional advantage, the high-rate cell **60B** can be sized (e.g., cell volume) to satisfy the requirements of the high-power output circuit **50**, without specific concern for powering the low-power control circuit **52**. As previously described, with prior art, single cell designs, cell volume is highly inefficient. The power source **54B** overcomes this problem by minimizing the size of the high-rate cell **60B**, and utilizing a more conveniently sized lower-rate cell **62B**. In other words, the high-rate cell **60B** can be a relatively simple shape that is conducive to coiled, serpentine, or other high-electrode area construction (but possibly with a lower volumetric energy density), whereas the lower-rate cell **62B** can be of a shape that conforms and efficiently utilizes a desired volumetric shape of the IMD **20**, such as a "D"-shaped pellet or bobbin cell with a relatively high volumetric energy density. The resulting power source **54B**, by virtue of its unique, complex shape, utilizes the volume available in the IMD **20** and thus contributes to the IMD **20** having an optimal volume.

[0056] Yet another alternative embodiment power source/circuitry configuration C is depicted in cross-section in **FIG. 5A**. More particularly, **FIG. 5A** shows a power source **54C** including a high-rate cell **60C**, a reservoir pellet **90**, and a lithium body **92** that serve as a lower-rate cell **62C**. The high-rate cell **60C**, the pellet **90**, and the lithium body **92** are disposed within a case **94** further containing an electrolyte **96**. Although not shown in **FIG. 5A**, the high-rate cell **60C** and the lower-rate cell **62C** (comprised of the reservoir cathode pellet **90** and the lithium body **92**) are connected in parallel to the high-power output circuit **50** (**FIG. 2**) and the low-power control circuit **52** (**FIG. 2**) by circuitry (not shown) that may or may not include a switch. Further, the lithium body **92** is approximately the same length and width as the cathode reservoir pellet **90**.

[0057] The high-rate cell **60C** can assume a number of constructions, but preferably includes a coiled anode **98** and cathode **100**. For example, the anode **98** is preferably a lithium material, whereas the cathode **100** is an appropriate metal-containing material (e.g., a metal oxide or metal sulfide), preferably SVO. Regardless, the anode **98** and the cathode **100** are preferably wound about the reservoir pellet **90**. Alternatively, the reservoir pellet **90** and the lithium body **92** can be positioned outside of the winding of the high-rate cell **60C**, as shown, for example, by the alternative embodiment of **FIG. 5B**.

[0058] Returning to **FIG. 5A**, the reservoir pellet **90** is of the same composition as the cathode **100**. For example, in a preferred embodiment, the reservoir pellet **90** is a dense SVO or MnO_2 cathode pellet. Similarly, the lithium body **92** is of the same composition as the anode **98**, and serves to balance the capability of the reservoir pellet **90**. In this regard, the lithium body **92** need not be a separate element, but instead, an inner-most turn **102** of the anode **98** (i.e., surrounding the reservoir pellet **90**) can be thickened (i.e., provided with additional lithium material).

[0059] The power source/circuitry configuration C provides the power source **54C** with a higher energy density than a conventional parallel plate or coil configuration by

utilizing the reservoir pellet **90** to charge the high-rate cell **60C** without the difficulties of fabricating, coiling, or folding multiple thick electrodes.

[0060] During use, the high-rate cell **60C** and the reservoir pellet **90** operate in parallel to power the low power control circuit **52** (**FIG. 2**). During a transient high-pulse operation, the high-rate cell **60C** and the reservoir pellet **90** operate to power the high-power output circuit **50** (**FIG. 2**). Most of the power is delivered by the high-rate cell **60C** due to its low internal resistance as compared to the lower-rate cell **62C** (again, defined by the reservoir cathode pellet **90** and the lithium body **92**). Following transient high-pulse operation, the lower-rate cell **62C** preferably acts to recharge the high-rate cell **60C** as previously described with respect to the power source **54B** (**FIG. 4**). In particular, the reservoir pellet **90** serves as an auxiliary cathode, accepting electrons and lithium ions from the cathode **100** following the transient high-pulse operation. For example, where the reservoir pellet **90** is comprised of a material that is chemically compatible with the composition of the cathode **100** (e.g., SVO or MnO_2), as the high-rate cell **60C** is discharged, the cathode **100** is charged or oxidized by the flow of electrons and lithium ions between the cathode **100** and the reservoir pellet **90**. The resulting power source **54C** has a higher average voltage, a higher volumetric energy density and an improved end of life voltage signal than a similar cell without the reservoir pellet **90**. Further, the lithium body **92** balances the capacity of the reservoir pellet **90**, thereby promoting recharging of the high-rate cell **60C** following a transient high power pulse.

[0061] In one more preferred embodiment of the power source **54C**, the high-rate cell **60C** and the lower-rate cell **62C** (or the reservoir pellet **90**) are connected in parallel, internal to the power source **54C** itself. For example, **FIG. 5C** illustrates one interconnection technique associated with the configuration C of **FIG. 5A**. As a point of reference, a portion of the case **94** has been removed to better illustrate component interconnection. With this in mind, the power source **54C** further includes a first conductive tab **102**, a second conductive tab **104**, and a connector **106**. The first tab **102** is connected to and extends from the cathode **100** associated with the high-rate cell **60C**. Conversely, the second tab **104** is connected to and extends from the reservoir (or cathode) pellet **90** forming the lower-rate cell **62C**. Finally, the connector **106** interconnects the tabs **102**, **104**, and terminates in a feed through pin **108** otherwise extending outwardly from the battery case **94**.

[0062] By internally connecting the cells **60C** and **62C** in parallel, only a single one of the feedthroughs **108** is required, thereby reducing the costs and complexities of other dual batter designs in which two or more feedthroughs are required. It will be understood that the construction of **FIG. 5C** necessitates that the cells **60C**, **62C** are not independently dischargeable, and a switch, such as the switch **80** of **FIG. 4** is not available. However, the design promotes shape flexibility and volumetric efficiency. For example, one particular manufacturing concern associated with high-energy IMD power supplies is the requirement, due to known safety concerns, of a wound cell utilizing a thick cathode. Where a wound design is employed, the thick cathode material tends to crack in the corners and transmits stress through other components (such as a separator plate and/or lithium anodes). This may, in turn, lead to internal shorts. With the configuration of **FIG. 5C**, however, a substantial fraction of the energy supply is stored in the reservoir pellet **90** (or lower rate cell **62C**), and the adjacent lithium body **92**. The pellet **90** is not wound, and thus can be relatively thick without presenting the stress concerns associated with a wound cathode material. Because a substantial fraction of the energy is stored in the pellet **90**, the cathode **100** material associated with the high-rate cell **60C** can now be relatively thin, and thus more readily wound without experiencing stress-related defects. Further, by forming the reservoir pellet **90** to be relatively thick, a radius of the inner most winding associated with the high rate cell **60C** is increased or greater than that found with conventional wound cells, again reducing winding-caused stress.

[0063] Yet another alternative power source/circuitry configuration D having enhanced volumetric efficiency is depicted schematically in **FIG. 6**. The configuration D includes a power source **54D** and circuitry **56D**. The power source **54D** includes a case **110** maintaining a high-rate cell **60D**, a lower-rate cell **62D**, and an electrolyte (not shown). The circuitry **56D** connects the cells **60D**, **62D** in parallel with the high-power output circuit **50** (**FIG. 2**) and the low-power control circuit **52** (**FIG. 2**). Although illustrated schematically in **FIG. 6**, the high-rate cell **60D** can assume any of the forms previously described and is preferably of a simple shape such that is conducive to assuming a coiled, serpentine, or other high-surface area electrode configuration. Conversely, the lower-rate cell **62D** is a relatively low-surface area auxiliary electrode assuming an irregular shape, such as a D-shape, otherwise conforming and efficiently utilizing an available volume of the case **110**. Once again, the lower-rate cell **62D** can be comprised of any of the material(s) previously described, and can be a medium- or low-rate cell. Regardless, the resulting power source **54D**, by virtue of its unique, complex shape, utilizes the volume available in the IMD **20** (**FIG. 1**) and thus contributes to an optimally sized device.

[0064] In operation, the power source **54D** operates similar to previous embodiments, with the high-rate cell **60D** and the lower-rate cell **62D** operating in parallel to power the high-power output circuit **50** (**FIG. 2**) and the low-power control circuit **52** (**FIG. 2**). In this regard, the circuitry **56D** associated with the power source **54D** may include a switch (not shown) that uncouples the high-rate cell **60D** from the low-power control circuit **52** during transient high power pulses. Operation of the lower-rate cell **62D** in isolation from the high-rate cell **60D** will continuously power the low-power control circuit **52** without concern for the voltage drop associated with the high-rate cell **60D**. Further, when the power source **54D** is subjected to a high-current pulse discharge, the high-rate cell **60D** and the lower-rate cell **62D** will equilibrate between pulses and thus stay at the same depth of discharge, with most of the capacity of the high-rate cell **60D** being discharged at a higher voltage than would be observed without the lower-rate cell **62D** connected in parallel.

[0065] Yet another, related alternative power source/circuitry configuration E having enhanced volumetric efficiency is depicted as part of an IMD **112** in **FIG. 7**. More particularly, the IMD **112** is shown as including a case **114**, a circuit **116** (shown generally in **FIG. 7**), and the power source **54E**. The power source **54E** includes a high-rate cell **60E** and a lower rate cell **62E**. With the embodiment of **FIG.**

7, the cells 60E, 62E are separately formed (i.e., separate enclosures) and are connected in parallel via circuitry 56E. Notably, the circuitry 56E does not include a switch, and the cells 60E, 62E are not independently dischargeable.

[0066] Though illustrated schematically in FIG. 7, the high-rate rate cell 60E can assume any of the forms previously described and is preferably of a simple shape, conducive to assuming a coiled, serpentine, or other high-surface area electrode configuration. Conversely, the lower-rate cell 62E is a relatively low-surface area auxiliary electrode shaped to efficiently utilize an available volume of the case 114. In one preferred embodiment, the high-rate cell 60E is a thin film battery known in the art. In this regard, one preferred method of manufacturing a thin electrode is to prepare a slurry of electrode material in an appropriate solvent. This slurry is then applied to a thin foil substrate as the current collector. To this end, the most common method is to use a "knife over roller" approach, whereby the slurry is applied to a moving web (e.g., the metal foil) using a knife edge to control thickness (i.e., a Doctor blade). The solvent is then evaporated leaving a thin film of cathode material. Alternatively, other known thin electrode manufacturing techniques are equally acceptable.

[0067] By forming the high rate cell 60E as a thin film battery, the power source 54E is characterized by an improved volumetric efficiency. Further, especially where the IMD 112 is an ICD, the power source 54E presents improved scaleability. As a point of reference, ICD batteries are typically built with maximum safe power capability (i.e., maximum safe electrode surface area). Thus, changing the size of a "standard" ICD battery in one dimension while maintaining a specific surface area typically imposes more geometric constraints than can be satisfied. As a result, for differently sized ICD applications, the "standard" ICD battery must often be changed in two dimensions, and therefore is not scaleable. The dual cell design of FIG. 7 overcomes this problem. In particular, by forming the high-rate cell 60E as a thin electrode allows the high-rate cell 60E to be located underneath the circuit 116. Conversely, the lower rate cell (preferably a medium-rate cell) 62E is constructed to have the same thickness as the internal dimensions of the case 114 (i.e., the same thickness as the circuits 116 and the high-rate cell 60E). As shown in FIG. 7, then, the lower rate cell 62E is positioned adjacent the circuit 116/high-rate cell 60E stack. The high-energy capacitors (not shown) of the ICD 112 are located on the other side of the lower-rate cell 62E and match the medium rate cell 62E in thickness. For a differently sized ICD, the lower rate cells 62E can be scaled in one dimension to provide the energy needs for a particular application. However, the circuit 116, the high-rate cell 60E, the capacitors, and any device connector blocks (not shown) are all fixed components that do not vary. Thus, the configuration of FIG. 7 meets desired scaleability criteria.

[0068] Another alternative embodiment power source/circuitry configuration F is depicted schematically in FIG. 8. The configuration F includes a power source 54F and associated circuitry 56F. Once again, the power source 54F includes a first, high-rate cell 60F and a second, lower-rate cell 62F. The circuitry 56F connects the high-rate cell 60F and the lower-rate cell 62F to the high-power output circuit 50 and the low-power control circuit 52. Unlike previous embodiments, the circuitry 56F need not necessarily connect the cells 60F, 62F in parallel. Further, while the lower-rate cell 62F is highly similar to previously described embodiments, the high-rate cell 60F is preferably an anode limited cell as described below.

[0069] In particular, for the configuration F, the high-rate cell 60F includes a solid cathode, liquid organic electrolyte and a lithium anode for delivering high current pulses. The cell 60F further includes a casing (not shown) containing the cell components and the cathode structure generally wound in a plurality of turns, with the lithium anode interposed between the turns of the cathode winding. The casing also contains a non-aqueous liquid organic electrolyte preferably comprising a combination of lithium salt and an organic solvent operatively contacting the anode and the cathode. An electrical connection is provided to the anode and an electrical connection is provided to the cathode. The cathode includes an active material such as SVO or MnO_2.

[0070] With the above-construction, the high-rate cell 60F is a volumetrically constrained system. The amounts of each component that goes into the cell 60F (cathode, anode, separator, current collectors, electrolytes, etc.) cannot exceed the available volume of the battery case. In addition, the appropriate amount of some components depends upon the amount of other components that are used. These components must be "balanced" to provide discharge to the extent desired.

[0071] For example, in a cathode limited Li/SVO battery such as is used in a defibrillator application, the capacity (Q_+) of the cathode must not exceed the capacity (Q_-) of the anode. The volume occupied by the other battery components also depends on the cathode capacity (Q_+) as reflected by the amount of cathode material in the battery. All of the battery components must be adjusted for a given battery volume.

[0072] Conventionally balanced lithium anode cells used with ICDs are balanced with sufficient lithium and electrolyte to discharge the cathode to completion. However, conventionally balanced cells have impedances that increase with time and depth-of-discharge. The power capability of these cells is limited by electrode area constraints imposed for safety reasons. Historically, it has been possible to use nearly the total capacity of the battery while maintaining adequate power (i.e., acceptable charge times). However, over time, conventionally balanced high-rate cells exhibit increased charge times due to increased cell impedance. When the cell can no longer satisfy charge time requirements, the ICD (or other IMD) must be replaced. To this end, industry standards have implemented more rigorous charge time requirements. Hence, it has become increasingly difficult to use the entire cell capacity before charge time failure.

[0073] One example of the above-described concern experienced by a Li/SVO type cell is illustrated graphically in FIG. 9. In particular, a conventional, Li/SVO high-rate cell design experiences a decrease in voltage over time as shown by curve 120. In addition, due to the increase in internal resistance over time results in an increasing capacitor charge time, as represented by the curve 122. As a point of reference, the curves 120, 122 extend from a beginning of life (BOL) point to an end of life (EOL) point. Just prior to EOL, manufacturers typically delineate a potential loss of function (indicated at "PLF" in FIG. 9) for the power source with respect to a particular IMD application. PLF is deter-

mined by circuit performance requirements of the IMD. For the example of **FIG. 9**, according to manufacturer standards, the conventionally balanced cell will experience a potential loss of function (PLF) at approximately 2.20 volts. To ensure that the IMD is explanted and replaced prior to PLF, industry standards require the IMD to provide an elective replacement indicator (ERI) to the user. The ERI is normally designated by the manufacturer with reference to the voltage curve **120** just prior to the PLF. For example, a manufacturer's standards may require that the IMD continue to operate for three months after ERI. With this standard in mind, the manufacturer works backwards from the PLF to select an ERI value that satisfies the so-selected standard. With reference to the example of **FIG. 9**, a common ERI value is 2.45 volts.

[0074] With the above definitions in mind, **FIG. 9** illustrates graphically that the charge time curve **122** is dependent upon depth-of-discharge or time, increasing from BOL to both ERI and PLF. Due to this time dependence, and as a point of reference, the charge time for a typical high-rate cell useful with an IMD is approximately 8 seconds at BOL, 14 seconds at ERI, and 25 seconds at PLF. As IMD performance requirements continue to evolve, it is highly likely that charge times in excess of 16 seconds may no longer be acceptable. In other words, future industry requirements may require a PLF value of 16 seconds (and thus a correspondingly decreased ERI value). While an IMD incorporating a lithium-based high-rate cell can be programmed to provide an earlier ERI signal (relative to the charge time curve **120**), due to the dependence upon depth-of-discharge or time, only a small portion of the battery's capacity will be used at this reduced ERI level. For example, at ERI corresponding with a charge time of 12 seconds, approximately 40% of a conventional cell's capacity has been used. Obviously this low efficiency is highly undesirable.

[0075] To overcome the time-dependent characteristics associated with previous lithium-based high-rate cells, the power source **54F (FIG. 8)** forms the high-rate cell **60F (FIG. 8)** to be anode limited. In particular, the high-rate cell **60F** is preferably a lithium limited cell as described, for example, in U.S. Pat. No. 5,458,997, the teachings of which are incorporated herein by reference. Generally speaking, available lithium-based high-rate cells, such as Li/SVO, Li/MnO_2, etc., are re-balanced such that the cell contains sufficient lithium and electrolyte to be discharged only to a first voltage plateau (labeled as **124** in **FIG. 9**). The volume made available by using less lithium and electrolyte allows more room for cathode material, thereby extending the first voltage plateau as shown by the dotted line **126**. With this configuration, the lithium anode is depleted prior to cathode depletion, thereby prohibiting the formation of gas. In addition, the lithium limited design generates minimal impedance over a majority of the battery's life. In one preferred embodiment, the lithium limited, high-rate cell **60F** is a SVO/CF_x hybrid cathode design, where x is in the range of 0.9-1.1.

[0076] As illustrated graphically in **FIG. 10**, the lithium limited high-rate cell **60F (FIG. 8)** exhibits charge time characteristics that have little dependence upon depth-of-discharge or time. As a point of reference, **FIG. 10** depicts a voltage curve **130** and a charge time curve **132**. As compared to the conventionally balanced cell performance characteristics illustrated in **FIG. 9**, the voltage curve **130** of the lithium limited high-rate cell **60F** has an extended first voltage plateau **134**, and a rapid voltage decrease after the second voltage plateau **136**. Importantly, however, prior to a second voltage plateau **136**, the charge time curve **132** increases only slightly, if at all, with increased depth-of-discharge and/or time. Effectively, then, the lithium limited high-rate cell **60E** is characterized by a rate capability that exhibits minimal dependence on time or depth-of-discharge throughout a majority of the battery's life. With this characteristic in mind, an IMD incorporating the power source **54F (FIG. 8)** including the high-rate cell **60F** can be programmed to establish the PLF and ERI values shown in **FIG. 10**.

[0077] By way of example, and in accordance with one preferred embodiment, the PLF is established at approximately 2.6 volts and the ERI at 2.65 volts. At these values, the rate capability or charge time curve **132** exhibits minimal dependence upon depth-of-discharge and time. For example, the BOL charge time is approximately 8 seconds, the ERI charge time is approximately 10 seconds, and the PLF charge time is approximately 16 seconds. Following the second voltage plateau **136**, the charge time rapidly increases to EOL. However, unlike conventionally balanced cells, the ERI and PLF of the anode limited high-rate cell **60F** are relatively close to the EOL (relative to an overall length of the voltage curve **130**). Thus, unlike conventionally balanced high-rate cells, the anode limited high-rate cell **60F** allows for selection of an ERI value at which rate capability and charge time has minimal dependence upon depth-of-discharge or time, and results in a large portion of the cell's **60F** capability being utilized. More particularly, the ERI of the high-rate cell **60F** is selected such that at least 40 percent of the cathode is consumed; preferably at least 50 percent; more preferably at least 60 percent; most preferably at least 75 percent.

[0078] As previously described, with embodiment F **(FIG. 8)**, the high-rate cell **60F** and the lower-rate cell **62F** need not necessarily be connected in parallel. However, with parallel wiring, the lower-rate cell **62F** will effectively recharge the high-rate cell **60F** following a transient high power pulse, according to the recharging mechanism previously described. Further, with the parallel configuration, it is preferred that the lower-rate cell **62F** be designed to have a higher voltage (beyond BOL) than the high-rate cell **60F** such that as the cells **60F, 62F** are discharged, the high-rate cell **62F** will remain nearer its BOL voltage and rate capability through more of the cell's **60F** useful life. In an even further preferred embodiment of configuration F employing a parallel construction, the high-rate cell **60F** is a lithium-limited SVO cell and the lower-rate cell **62F** is a SVO/CF_x hybrid cathode low-rate cell. This construction provides both of the cells with similar BOL voltages, similar depletion voltages (e.g., greater than 90% depletion at PLF), and the lower-rate cell **62F** will have a higher voltage (beyond BOL) than the high-rate cell 60F.

[0079] The IMD with dual cell power source of the present invention provides a marked improvement over previous designs. In one embodiment, by connecting a first, high-rate cell and a second, lower-rate cell in parallel to a control circuit and an output circuit, and including a switch to selectively uncouple the high-rate cell and the control circuit, the IMD will efficiently utilize the capacity in both cells independent of charge conditions. Regardless of whether the

switch is included, the preferred parallel connection can facilitate the lower-rate cell recharging the high-rate cell following a transient high power pulse depending upon a construction of the high-rate cell. In another alternative embodiment, the dual cells are provided as a single reservoir. In yet another alternative embodiment, the high-rate cell has an anode-limited construction and exhibits a charge time characteristic that has minimal dependence on time or depth-of-discharge. With this configuration, a majority of the high-rate cell's capacity is utilized while satisfying rigorous charge time requirements.

[0080] **FIG. 11** illustrates an implantable medical device (IMD) **200** in accordance with another embodiment of the present invention. The IMD **200** according to this embodiment may be provided in the form of a pacemaker, cardioverter, defibrillator, neural stimulator, or drug administering device. It will be appreciated, however, that the IMD **200** may take the form of various other implantable medical devices, and, thus, need not necessarily be limited to the aforementioned examples. For purposes of illustration, however, the IMD **200** will be described in the configuration of an implantable cardiac defibrillator (ICD).

[0081] According to the illustrated embodiment, the IMD **200** comprises a control circuit **205** that controls the overall operation of the IMD **200**. The control circuit **205** may be configured to monitor physiological data via one or more electrodes disposed within the patient's body, which are coupled to the IMD **200** via electrical leads. For example, the control circuit **205** may monitor cardiological activity via one or more electrodes implanted within the patient's heart. The control circuit **205** may collect and process the physiological data received via the implanted electrodes. Depending on the physiological data received at the IMD **200** via the implanted electrodes, the control circuit **205** may further be configured to deliver a therapy to a part of the patient's body. In accordance with the exemplary embodiment, the therapy may be provided in the form of a therapeutic electric pulse that is delivered to the patient's heart via the one or more electrodes implanted within the heart.

[0082] In accordance with one embodiment of the present invention, the control circuit **205** is provided in the form of a processor unit **207**, as shown in **FIG. 11A**, to control the overall operation thereof. In one embodiment, the processor unit **207** may, for example, take the form of a microprocessor, a microcontroller, or a digital signal processor. The control circuit **205** may further include a memory module **208** for storing the physiological data that is received by the one or more electrodes implanted within the patient's body. The memory module **208** may also store software firmware, and/or microcade that executes on the processor unit **207** for controlling the IMD **200**.

[0083] Referring again to **FIG. 11**, the IMD **200** may further include a high power output circuit **210** for delivering an electrical pulse therapy, such as a defibrillation or cardioversion/defibrillation pulse in accordance with the exemplary embodiment. The high power output circuit **210** may be provided in the form of a capacitor (not shown) for generating a high output electronic pulse that is delivered to the patient's heart via the one or more electrodes that are implanted therein. According to the illustrated embodiment, the high power output circuit **210** may receive a control signal from the control circuit **205** to deliver the high output electric shock in response to the analysis of the physiological data (i.e., electric cardiac signals) received via the one or more electrodes implanted within the patient's heart.

[0084] In accordance with the illustrated embodiment, the IMD **200** is further provided with a communication interface circuit **215**, which may provide communication capabilities for the IMD **200** to communicate with an external data processing device. The data processing device may be configured to monitor and/or analyze the physiological data that is collected and subsequently transmitted by the IMD **200**. It will be appreciated, however, that the communication interface circuit **215** may also be configured to communicate with various other devices that are external to the patient's body without departing from the spirit and scope of the present invention. In an alternative embodiment, the communication interface circuit **215** may communicate with a transmitting device (not shown) that is external to the IMD **200**, but within the patient's body. This transmitting device may then communicate with an external data processing unit.

[0085] According to the illustrated embodiment, the communication interface circuit **215** is configured to communicate physiological data obtained by the control circuit **205** from the one or more electrodes implanted within the patient's body. The communication interface circuit **215** may also be configured to receive data that is generated by another device externally from the IMD **200** that is to be processed by the control circuit **205**. According to the illustrated embodiment, the communication interface circuit **215** communicates data with the external device via wireless communication.

[0086] In accordance with the illustrated embodiment, the IMD **200** is further configured with a power source **220** to provide electrical power to the control circuit **205**, high power output circuit **210** and the communication interface circuit **215**. The power source **220** inherently plays a significant role in the operation of the IMD **200** since the IMD may enter of limited function mode as the battery approaches end-of-life. As such, the IMD may not be capable of delivering an appropriate therapy to the patient, thereby compromising the patient's health. Moreover, because the IMD **200** is implanted within the patient's body, battery accessibility usually requires a surgical procedure. Accordingly, if the power source **220** fails, the patient's health may be placed in jeopardy until such procedure is performed.

[0087] Turning now to **FIG. 12**, the communication capabilities of the IMD **200** with an external device is shown in accordance with one embodiment of the present invention. The communication interface circuit **215** of the IMD **200** is configured with a wireless interface **230** for communicating through a wireless communication medium **232** to a data processing device **240** via a data transfer device **235**. In accordance with the illustrated embodiment, the wireless interface **230** may take the form of a radio frequency (RF) transceiver that transmits and receives radio frequency signals with the data transfer device **235**, which is also configured with an RF transceiver. It will be appreciated, however, that other forms of communication protocols may be utilized between the wireless interface **230** of the IMD **200** and the data transfer device **235** either in lieu of or in addition to radio frequency communication without depart-

ing from the spirit and scope of the present invention. For example, the communication protocol utilized between the wireless interface **230** and the data transfer device **235** may include ultrasound communication, among other types of communication.

[0088] According to the illustrated embodiment, the data transfer device **235** may be provided in the form of a hand-held device that may be proximately placed to the implantable medical device **200** implanted within the patient's body. In this embodiment, the data transfer device **235** is coupled to the data processing device **240** via a wired link **237**. It will be appreciated, however, that the data transfer device **235** may alternatively communicate with the data processing device **240** via a wireless communication medium. For example, the wireless communication medium between the data transfer device **235** and the data processing device **240** may be an RF communication medium or an infrared (IR) communication medium. Alternatively, in one embodiment, data transfer device **235** is eliminated, with the data transfer occurring directly between wireless interface **230** and data processing device **240**.

[0089] It will further be appreciated that the power level of the communication signals between the communication interface circuit **215** of the IMD **200** and the data transfer device **235** may vary as well. For example, low power RF communication may be used between the IMD **200** and the data transfer device **235** such that it may have to be placed within close proximity to the IMD **200**. Alternatively, a higher transmission power level may be used over the RF communication medium **232** such that close physical proximity of the data transfer device **235** and the IMD **200** is not necessary. Of course, it will be appreciated that the higher the transmission power level that is used over the RF communication medium **232**, the higher the drain on the power source **220** of the IMD **200**.

[0090] As previously mentioned, the physiological data is collected by the control circuit **205** of the IMD **200** via the one or more implanted electrodes within the patient's body. In one embodiment, the physiological data may take the form of electrical cardiac signals from electrodes implanted within the patient's heart, and recorded within the memory module **208** of the IMD **200** in the form of an electrocardiogram, for example. The physiological data may subsequently be retrieved from the memory module **208** and transferred to the communication interface circuit **215** for wireless transmission to the data transfer device **235** for monitoring and/or processing by the data processing device **240**. In an alternative embodiment, the physiological data may be obtained by the control circuit **205** and transferred to the communication interface circuit **215** for transmission to the data transfer device **235** on a real-time basis as the data is sensed by the one or more implanted electrodes within the patient's body. In addition to the transmission of physiological data to the data processing device **240** via the data transfer device **235**, the communication interface circuit **215** may also transmit data relating to the performance of the IMD **200**. The performance data may include, for example, the effectiveness of a previously delivered therapy from the IMD **200** to the patient's body.

[0091] In accordance with one embodiment of the present invention, the data processing device **240** is provided in the form of a programmer or other computer. The data processing device **240** may be used to monitor and/or analyze the physiological data and/or performance data transmitted from the IMD **200** via the communication interface circuit **215**. The data processing device **240** may also determine the efficiency of the therapy that is delivered by the IMD **200** based upon the physiological data and performance data collected. For example, the data processing device **240** may be used to determine whether the therapy delivered to the patient was of a proper energy intensity.

[0092] Based upon the analysis performed by the data processing device **240** using the physiological and performance data that was received by the IMD **200**, the data processing device **240** may also be configured to transmit programming data to the IMD **200** via the data transfer device **235** to adjust various settings of the IMD **200**. For example, if it is determined by the data processing device **240** that the IMD **200** is delivering a higher intensity of an electric pulse therapy signal than is necessary (based upon the physiological data collected, for example), the programming data transmitted to the IMD **200** may reduce the intensity of the electric therapy signal delivered to the patient's body.

[0093] Typically, the communication interface circuit **215** of the IMD **200** requires relatively high current pulses, thus resulting in a relatively higher drain from the power source **220**. If a substantial amount of data is communicated between the communication interface circuit **215** and the data transfer device **235**, it may create a significant drain on the power source **220** because of the high current pulses and the amount of time the communication interface circuit **215** is transmitting data. Additionally, as the amount of data communicated between the IMD **200** and the data transfer device **235** increases, the burden placed on the power source **220** is also increased, thereby decreasing the life of the power source **220** within the IMD **200**.

[0094] Turning now to **FIG. 13**, a more detailed representation of the power source **220** is provided according to one embodiment of the present invention. The power source **220** comprises a primary power source **250** and a secondary power source **255**. The primary power source **250** is used to power the control circuit **205** of the IMD **200**, as well as the high-output power circuit **210**. In accordance with one embodiment of the present invention, the primary power source **250** takes the form of a lithium/CFx—CSVO battery. It will be appreciated, however, that the primary power source **250** may take the form of various other battery types, which may include Li/CSVO, Li/CF_x, Li/MnO_2, Li/I2, Li/$SOCl_2$, or other similar type chemistries.

[0095] In accordance with the illustrated embodiment, the secondary power source **255** provides power to the communication interface circuit **215** to alleviate any additional burden that the communication interface circuit **215** would have placed on the primary power source **250**. In accordance with one embodiment, the secondary power source **255** is provided in the form of a rechargeable battery. The secondary power source **255** may comprise a lithium-ion battery with either a liquid or polymer electrolyte. It will be appreciated, however, that the secondary power source **255** may also take the form of other battery types, such as nickel/metal hydride or other similar type chemistries without departing from the spirit and scope of the present invention. According to the illustrated embodiment, the secondary

power source **255** may be recharged via a transcutaneous magnetic induction process, as is well established in the art.

[0096] In accordance with one embodiment, the secondary power source **255** powers only the communication interface circuit **215**, thereby relieving the burden of additional power requirements that the communication interface circuit **215** would require from the primary power source **250**. Thus, in this embodiment, the secondary power source **255** is a dedicated power source for the communication interface circuit **215**. Accordingly, the primary power source **250** needs to provide power only to the essential "life-support" operating circuitry of the control circuit **205** and the high-output power circuit **210** without the need to provide power to support the IMD **200**'s communication requirements (i.e., through the communication interface circuit **215**), thereby conserving the power and life of the primary power source **250**. The primary power source may take the form of any of the dual-cell embodiments discussed above. Alternatively, the primary power source may be a conventional, single-cell design.

[0097] In the illustrated embodiment of **FIG. 13**, the power sources **250** and **255** may operate independently of each another. Thus, in one embodiment, if one of the power sources **250, 255** fails, the other power source **250, 255** continues to power its respective circuit(s).

[0098] Turning now to **FIG. 14**, the power source **220** is shown in accordance with another embodiment of the present invention. In this particular embodiment, the primary power source **250** and the secondary power source **255** are coupled to a power source switch **260**, which is capable of switching connections to provide power to the various components of the IMD **200**. As mentioned with the configuration provided in **FIG. 13**, the primary power source **250** ordinarily provides power only to the control circuit **205** and the high output power circuit **210** of the IMD **200**. The secondary power source **255**, on the other hand, ordinarily provides power only to the communication interface circuit **215**. In accordance with the illustrated embodiment of **FIG. 14**, the power source switch **260** is configured to switch connections of the primary power source **250** and/or the secondary power source **255** depending on whether or not the power sources **250, 255** are depleted of their power.

[0099] In accordance with one embodiment, the switch **260** is coupled to a power level sensor **265**, which is configured to determine the remaining power level of the primary power source **250** and/or secondary power source **255**. The power level sensor **265** may be further configured to determine whether the remaining power level of the primary and/or secondary power sources **250, 255** has fallen below a predetermined power level. Accordingly, the power source switch **260** may be configured to switch connections between the circuits **205, 210,** and **215** of the IMD **200** and the primary and secondary power sources **250, 255** based upon the power level being below the predetermined threshold value as determined by the sensor **265**. In one embodiment, the predetermined threshold value may be a power level just above a remaining power level of zero (i.e., a dead battery).

[0100] For example, if the IMD **200** is transferring data between its communication interface circuit **215** and the data transfer device **235** (**FIG. 2**), and the power level sensor **265** determines that the power level of the secondary power source **255** is nearly depleted (i.e., below a predetermined threshold), the sensor **265** may send a control signal to the switch **260** to couple the primary power source **250** to the communication interface circuit **215** of the IMD **200** so as not to disrupt the data transfer. Similarly, if the power level within the primary power source **250** is determined to be depleted below a predetermined threshold, the power source switch **260** may switch the connections of the control circuit **205** and/or high output power circuit **210** to receive power from the secondary power source **255**, as opposed to receiving power from the primary power source **250**.

[0101] In an alternative embodiment, the power source switch **260** may include the circuitry to sense the power level remaining within the primary power source **250** and/or the secondary power source **255**, and to switch connections between the circuits **205, 210,** and **215** of the IMD **200** and the primary and secondary power sources **250, 255** based upon the sensed power levels. That is, the sensor **265** for sensing the remaining power level of the primary and secondary power sources **250, 255** may be an integral component of the power source switch **260** as opposed to being a separate component as illustrated in **FIG. 14**.

[0102] Although the present invention has been described with reference to preferred embodiments, it will be appreciated by those of ordinary skill in the art that a wide variety of alternate and/or equivalent implementations calculated to achieve the same purposes may be substituted for the specific embodiments shown and described without departing from the spirit and scope of the present invention.

What is claimed is:

1. An apparatus, comprising:

first and second power sources;

a control circuit coupled to the first power source to control the operation of the apparatus, the control circuit being adapted to receive power from the first power source; and

a communication circuit coupled to the second power source to communicate with an external device, the communication circuit being adapted to receive power from the second power source.

2. The apparatus of claim 1, wherein the first power source comprises a battery.

3. The apparatus of claim 2, wherein the battery comprises at least one of a Li/CF_x—CSVO, Li/CSVO, Li/CF_x, Li/MnO_2, Li/I2, and $Li/SOCl_2$ battery.

4. The apparatus of claim 1, wherein the second power source comprises a rechargeable battery.

5. The apparatus of claim 4, wherein the rechargeable battery comprises at least one of a lithium-ion and a nickel/metal-hydride battery.

6. The apparatus of claim 1, further comprising:

a switch for coupling the first power source to the communication circuit upon occurrence of a first predetermined event.

7. The apparatus of claim 6, wherein the first and second power sources have a remaining power level associated therewith, the apparatus further comprising:

a sensor for sensing the remaining power level of at least one of the first power source and second power source.

8. The apparatus of claim 7, wherein the first predetermined event includes the sensor sensing the remaining power level of the second power source being below a remaining power level threshold.

9. The apparatus of claim 7, wherein the switch includes means to couple the second power source to the control circuit upon occurrence of a second predetermined event.

10. The apparatus of claim 9, wherein the second predetermined event includes the sensor sensing the remaining power level of the first power source being below a remaining power level threshold.

11. The apparatus of claim 1, wherein the control circuit further is adapted to obtain physiological data of a patient in which the apparatus is implanted.

12. The apparatus of claim 11, wherein the communication circuit includes means to transmit the physiological data to the external device.

13. The apparatus of claim 11, wherein the communication circuit includes means to receive programmed instructions from the external device.

14. The apparatus of claim 11, further comprising a high-power output circuit coupled to the central circuit to deliver a therapy to the patient depending on the physiological data obtained from the control circuit.

15. The apparatus of claim 14, wherein the high-power output circuit receives power from the first power source.

16. The apparatus of claim 15, wherein the first power source comprises a high-rate cell and a low-rate cell.

17. The apparatus of claim 15, wherein the high-rate cell provides power to the high-power output circuit and the low-rate cell provides power to the control circuit.

18. An implantable medical device, comprising:

a control circuit to control the operation of the implantable medical device and to obtain physiological data from a patient in which the implantable medical device is implanted;

a communication circuit coupled to the control circuit to transmit the physiological data to an external device;

a first power source coupled to the control circuit to provide power to the control circuit; and

a second power source coupled to the communication circuit to provide power to the communication circuit.

19. The implantable medical device of claim 18, wherein the first power source comprises a battery.

20. The implantable medical device of claim 19, wherein the battery comprises at least one of a Li/CF_x—CSVO, Li/CSVO, Li/CF_x, Li/MnO_2, Li/I_2, and Li/$SOCl_2$ battery.

21. The implantable medical device of claim 18, wherein the second power source comprises a rechargeable battery.

22. The implantable medical device of claim 21, wherein the rechargeable battery comprises at least one of a lithium-ion and a nickel/metal-hydride battery.

23. The implantable medical device of claim 18, further comprising:

a switch to couple the first power source to the communication circuit upon occurrence of a first predetermined event.

24. The implantable medical device of claim 23, wherein the first and second power sources each have a remaining power level associated therewith, the device further comprising:

a sensor coupled to the first and second power sources to sense the remaining power level of at least one of the first power source and second power source.

25. The implantable medical device of claim 24, wherein the first predetermined event includes the sensor sensing the remaining power level of the second power source being below a remaining power level threshold.

26. The implantable medical device of claim 24, wherein the switch couples the second power source to the control circuit upon occurrence of a second predetermined event.

27. The implantable medical device of claim 26, wherein the second predetermined event includes the sensor sensing the remaining power level of the first power source being below a remaining power level threshold.

28. A method for incorporating a power source in an implantable medical device, comprising the steps of:

providing power to a control circuit by a first power source, the control circuit obtaining physiological data of a patient in which at least the control circuit is implanted;

providing power to a communication circuit by a second power source; and

transmitting the physiological data from the communication circuit to an external device.

29. The method of claim 28, further comprising:

sensing a remaining power level of the second power source;

determining if the remaining power level has fallen below a predetermined threshold; and

providing power to the communication circuit by the first power source in response to determining that the remaining power level has fallen below the predetermined threshold.

30. The method of claim 28, further comprising:

sensing a remaining power level of the first power source;

determining if the remaining power level has fallen below a predetermined threshold; and

providing power to the control circuit by the second power source in response to determining that the remaining power level has fallen below the predetermined threshold.

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(54) **MATRICES WITH MEMORIES**

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(*) Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

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(57) **ABSTRACT**

Combinations, called matrices with memories, of matrix materials that are encoded with an optically readable code are provided. The matrix materials are those that are used in as supports in solid phase chemical and biochemical syntheses, immunoassays and hybridization reactions. The matrix materials may additionally include fluophors or other luminescent moieties to produce luminescing matrices with memories. The memories include electronic and optical storage media and also include optical memories, such as bar codes and other machine-readable codes. By virtue of this combination, molecules and biological particles, such as phage and viral particles and cells, that are in proximity or in physical contact with the matrix combination can be labeled by programming the memory with identifying information and can be identified by retrieving the stored information. Combinations of matrix materials, memories, and linked molecules and biological materials are also provided. The combinations have a multiplicity of applications, including combinatorial chemistry, isolation and purification of target macromolecules, capture and detection of macromolecules for analytical purposes, selective removal of contaminants, enzymatic catalysis, cell sorting, sensors and drug delivery, chemical modification and other uses. Methods for tagging molecules, biological particles and matrix support materials, immunoassays, receptor binding assays, scintillation proximity assays, non-radioactive proximity assays, and other methods are also provided. Sensors containing a memory in combination with a matrix are also provided.

20 Claims, 40 Drawing Sheets

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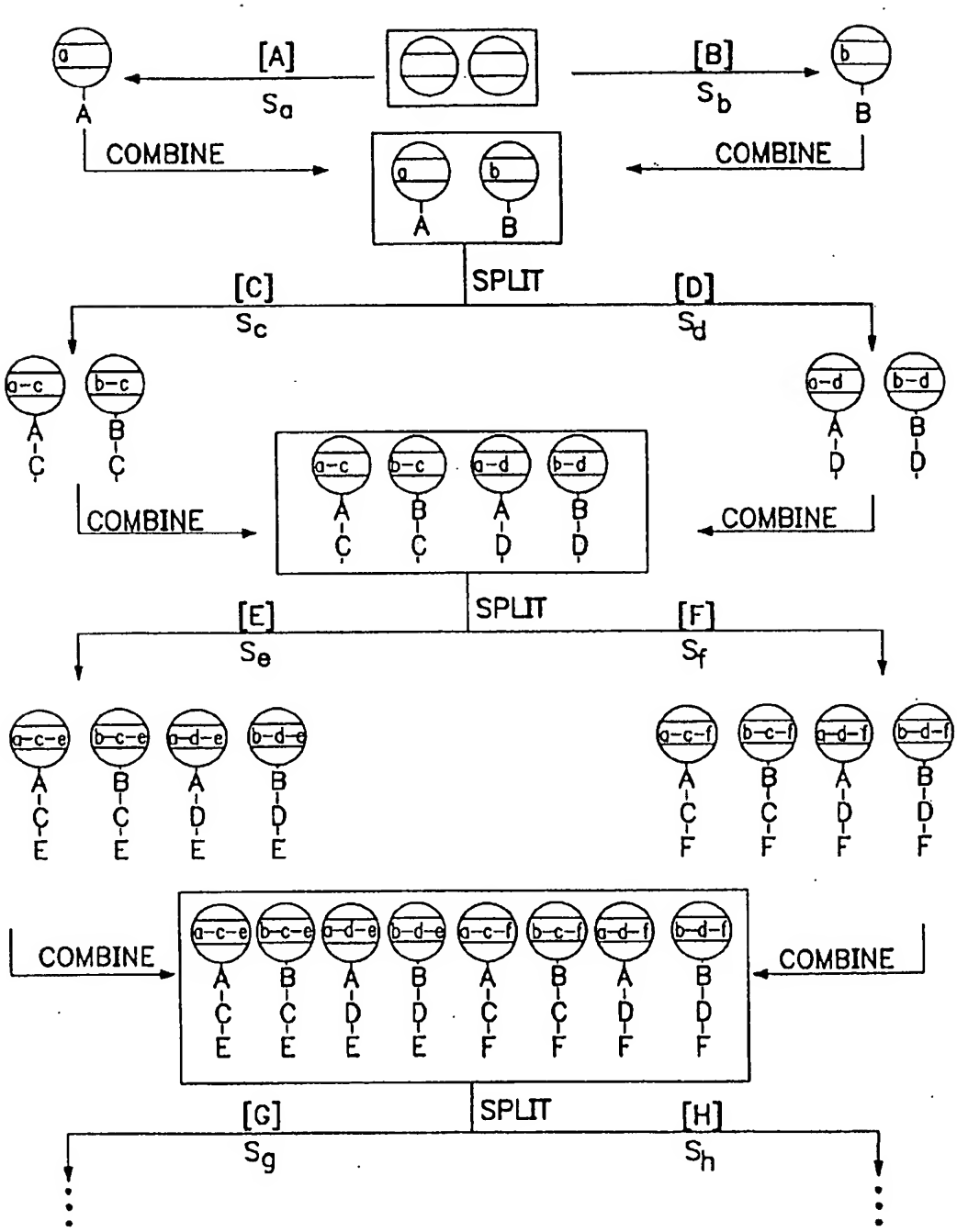

a
A
[A]
S_a
[B]
S_b
b
B
COMBINE
COMBINE
a
b
A
B
SPLIT
[C]
S_c
[D]
S_d
a-c
b-c
A C
B C
a-d
b-d
A D
B D
COMBINE
COMBINE
a-c
b-c
a-d
b-d
A C
B C
A D
B D
SPLIT
[E]
S_e
[F]
S_f
a-c-e
b-c-e
a-d-e
b-d-e
A C E
B C E
A D E
B D E
a-c-f
b-c-f
a-d-f
b-d-f
A C F
B C F
A D F
B D F
COMBINE
COMBINE
a-c-e
b-c-e
a-d-e
b-d-e
a-c-f
b-c-f
a-d-f
b-d-f
A C E
B C E
A D E
B D E
A C F
B C F
A D F
B D F
SPLIT
[G]
S_g
[H]
S_h

FIG. 1

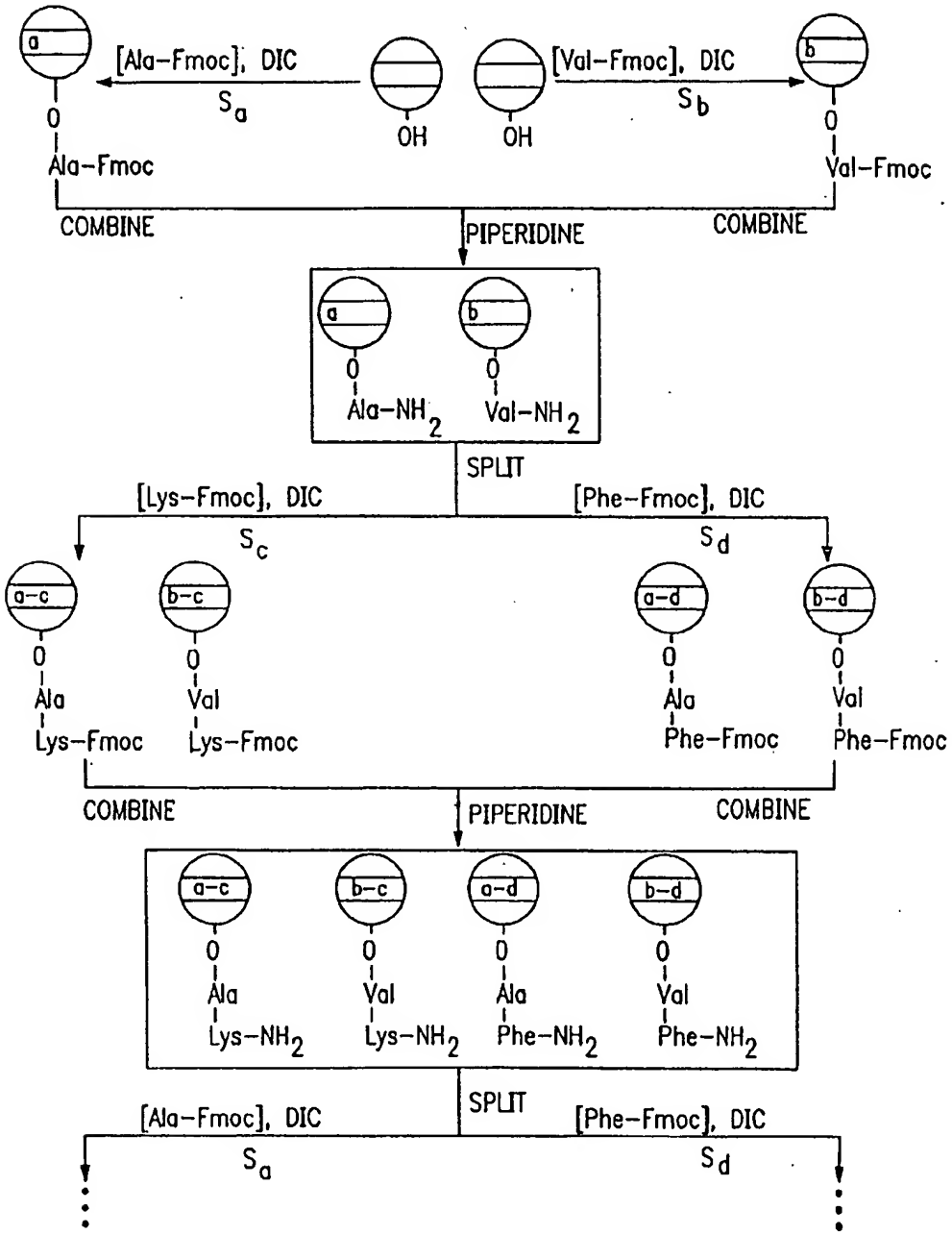

a
[Ala-Fmoc], DIC
S_a
OH
OH
[Val-Fmoc], DIC
S_b
b
O
Ala-Fmoc
O
Val-Fmoc
COMBINE
PIPERIDINE
COMBINE
a
b
O
Ala-NH_2
O
Val-NH_2
SPLIT
[Lys-Fmoc], DIC
S_c
[Phe-Fmoc], DIC
S_d
a-c
b-c
a-d
b-d
O
Ala
Lys-Fmoc
O
Val
Lys-Fmoc
O
Ala
Phe-Fmoc
O
Val
Phe-Fmoc
COMBINE
PIPERIDINE
COMBINE
a-c
b-c
a-d
b-d
O
Ala
Lys-NH_2
O
Val
Lys-NH_2
O
Ala
Phe-NH_2
O
Val
Phe-NH_2
SPLIT
[Ala-Fmoc], DIC
S_a
[Phe-Fmoc], DIC
S_d

FIG. 2

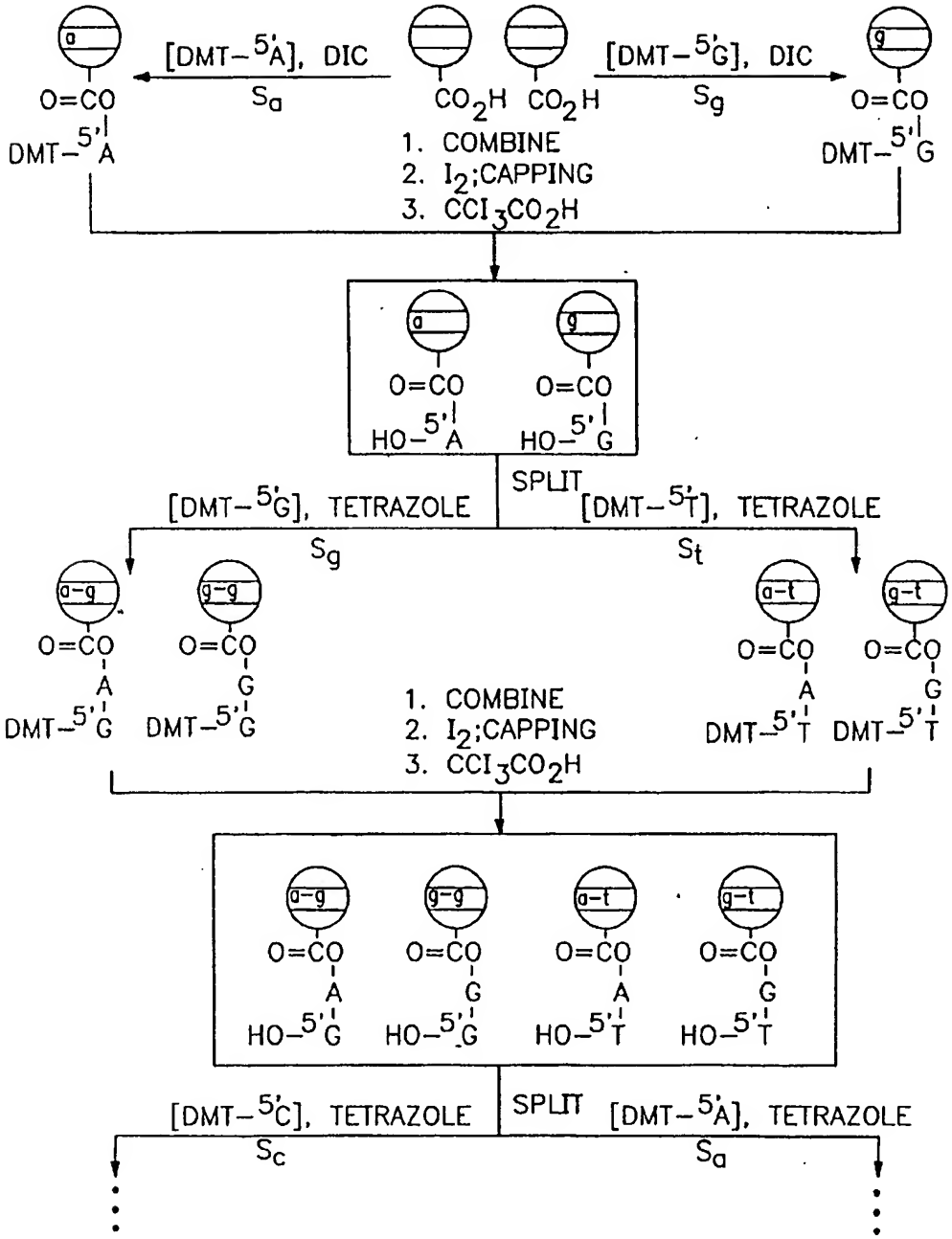

[DMT–5'A], DIC
Sa
[DMT–5'G], DIC
Sg
1. COMBINE
2. I2;CAPPING
3. CCl3CO2H
[DMT–5'G], TETRAZOLE
Sg
SPLIT
[DMT–5'T], TETRAZOLE
St
1. COMBINE
2. I2;CAPPING
3. CCl3CO2H
[DMT–5'C], TETRAZOLE
Sc
SPLIT
[DMT–5'A], TETRAZOLE
Sa

FIG. 3

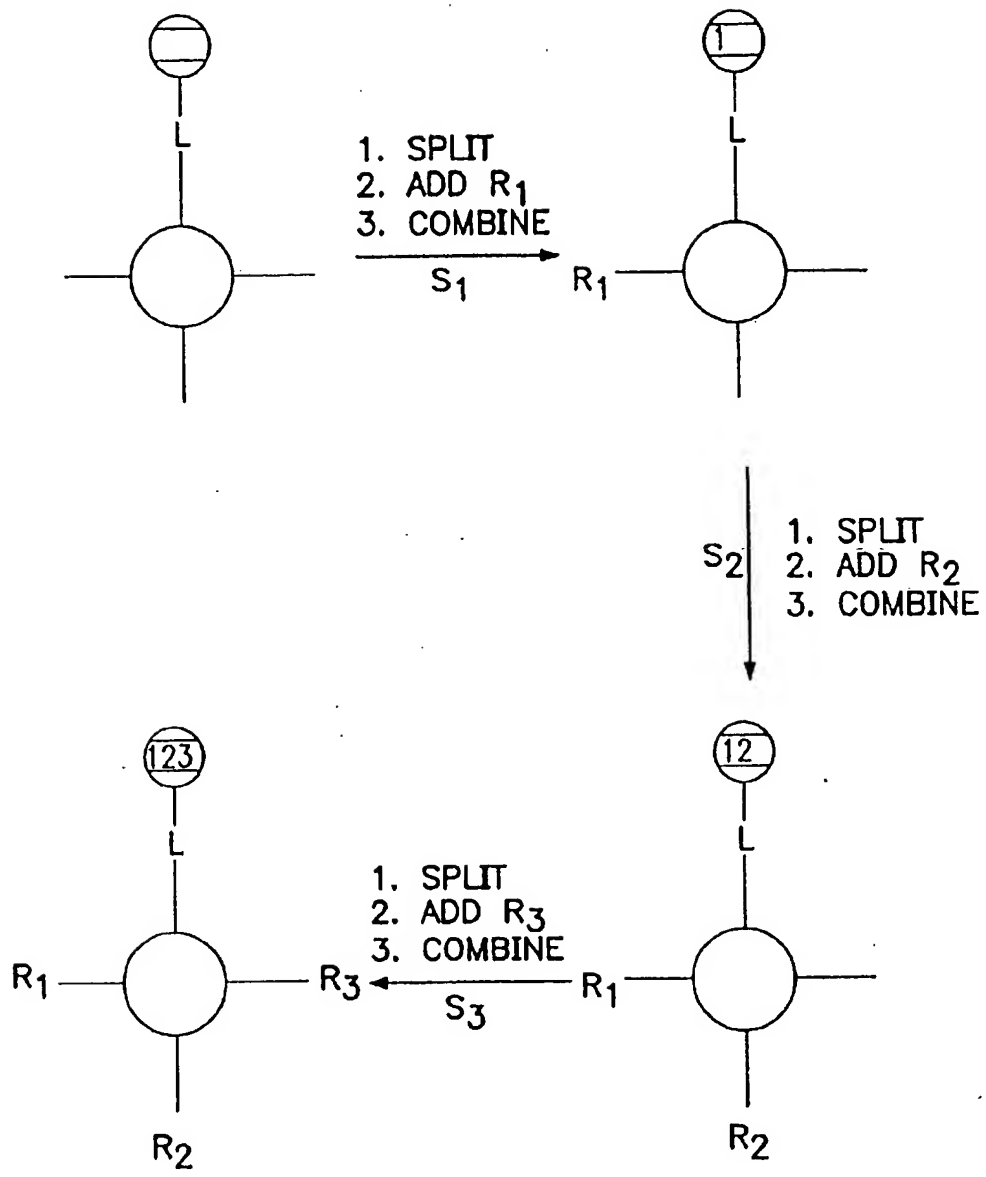

L
1. SPLIT
2. ADD R_1
3. COMBINE
S_1
R_1
1
L
S_2
1. SPLIT
2. ADD R_2
3. COMBINE
123
L
R_1
R_3
R_2
1. SPLIT
2. ADD R_3
3. COMBINE
S_3
R_1
12
L
R_2

FIG. 4

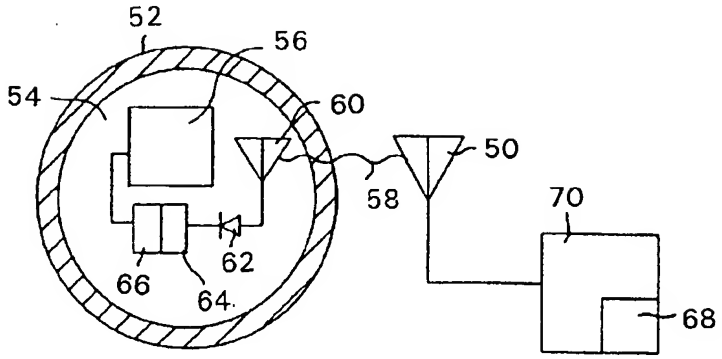

52
56
54
60
50
58
70
62
66
64
68

FIG. 5

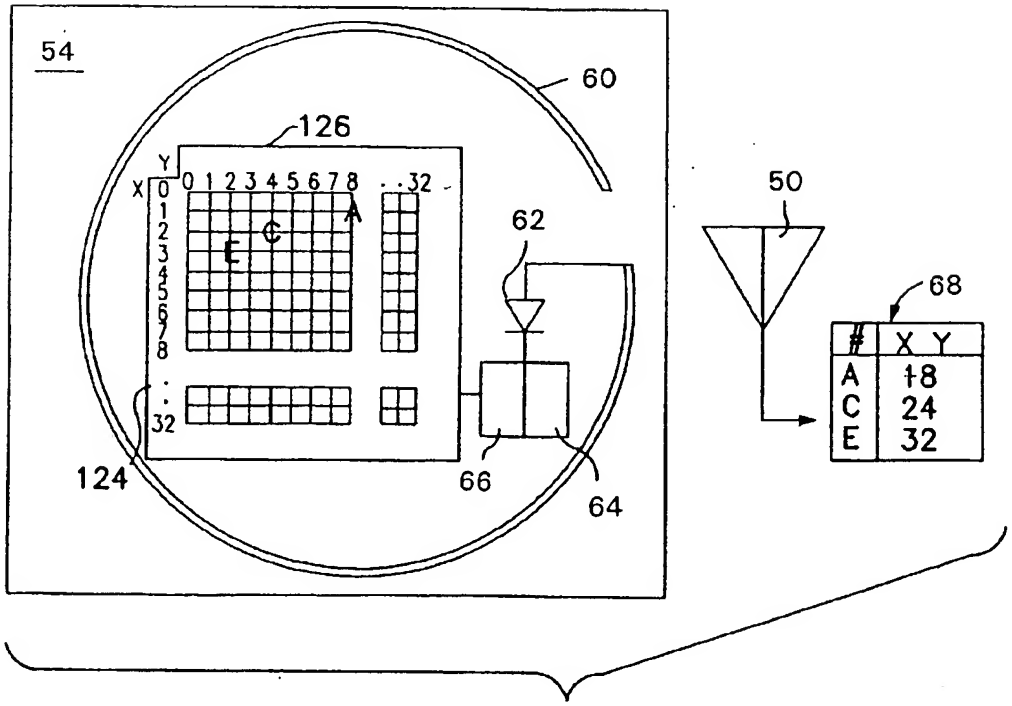

54
60
126
Y
X
0 1 2 3 4 5 6 7 8 . . 32
A
C
E
62
50
68
X Y
A 8
C 24
E 32
124
66
64

FIG. 6

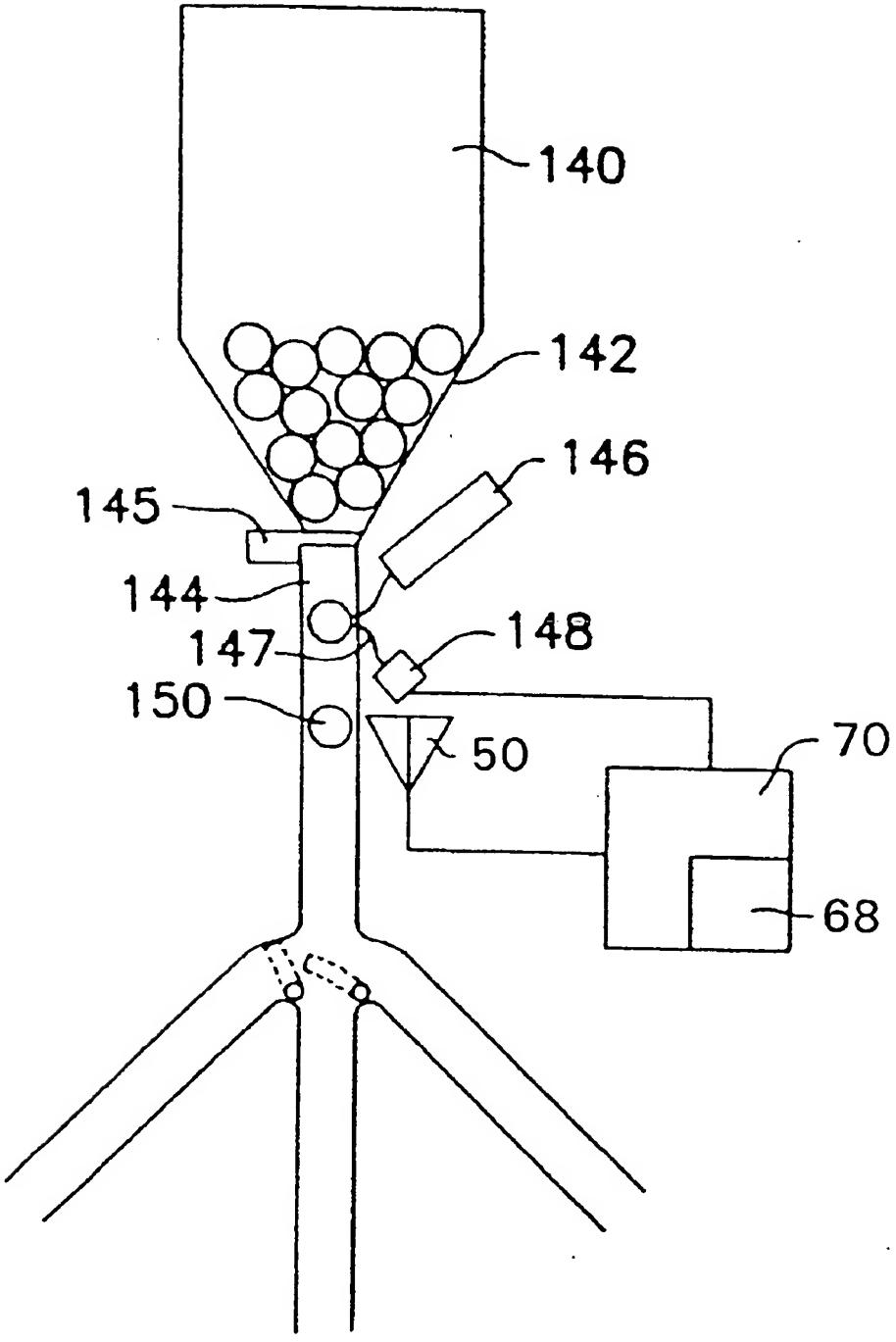


FIG. 7

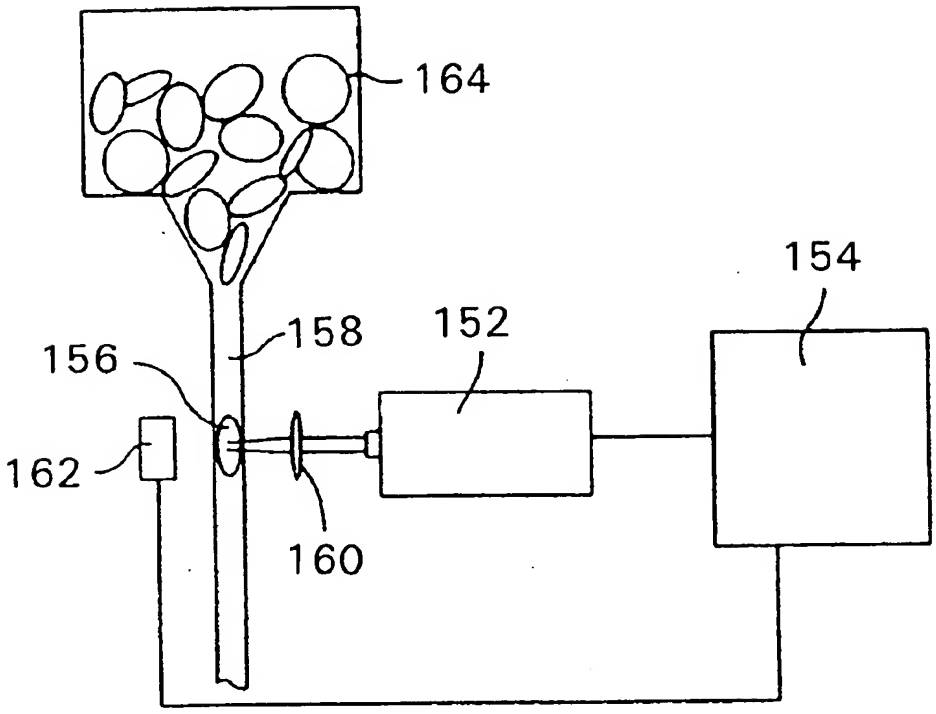


FIG. 8

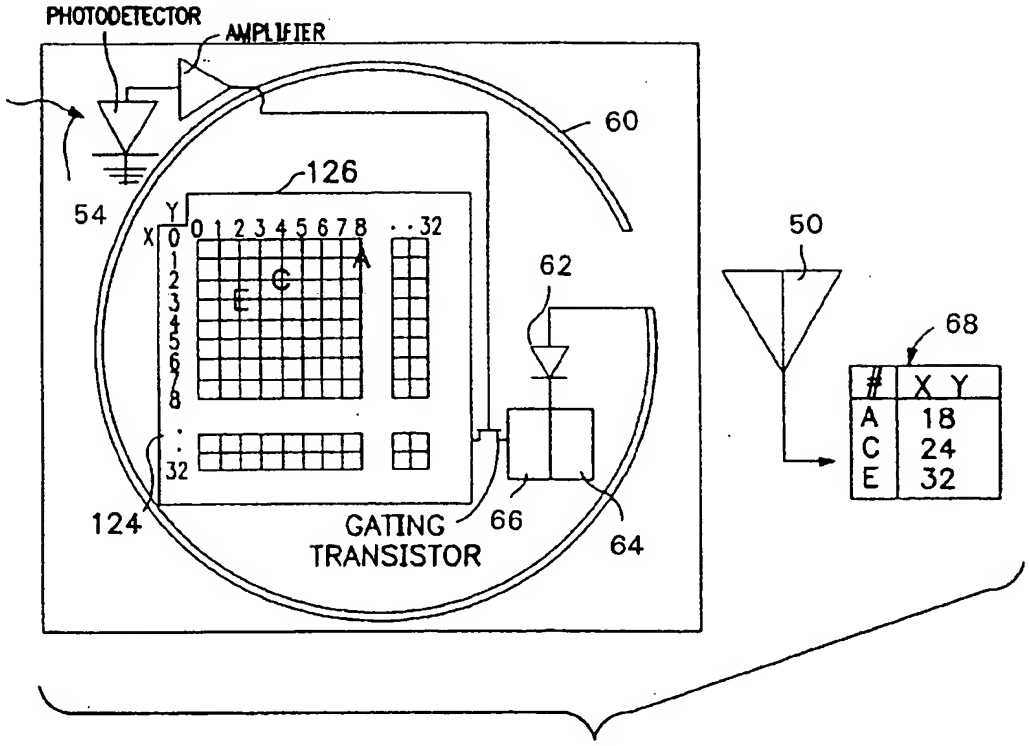

PHOTODETECTOR
AMPLIFIER
60
126
54
Y
X
0 1 2 3 4 5 6 7 8 . . 32
A
C
E
50
62
68
X Y
A 18
C 24
E 32
124
GATING TRANSISTOR
66
64

FIG. 9

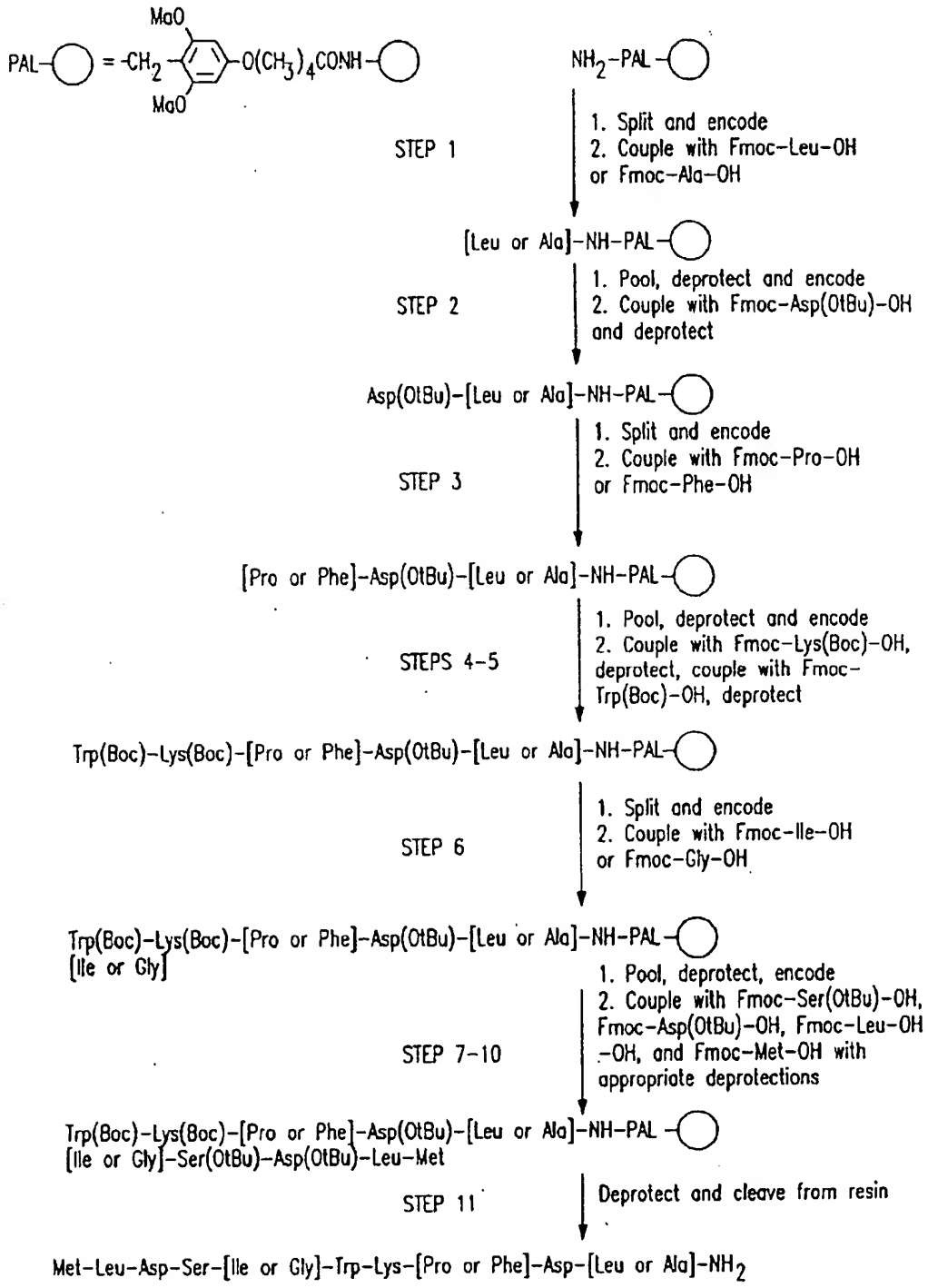

MaO
PAL = -CH2 - -O(CH3)4CONH-
MaO
NH2-PAL
STEP 1
1. Split and encode
2. Couple with Fmoc-Leu-OH
or Fmoc-Ala-OH
[Leu or Ala]-NH-PAL
STEP 2
1. Pool, deprotect and encode
2. Couple with Fmoc-Asp(OtBu)-OH
and deprotect
Asp(OtBu)-[Leu or Ala]-NH-PAL
STEP 3
1. Split and encode
2. Couple with Fmoc-Pro-OH
or Fmoc-Phe-OH
[Pro or Phe]-Asp(OtBu)-[Leu or Ala]-NH-PAL
STEPS 4-5
1. Pool, deprotect and encode
2. Couple with Fmoc-Lys(Boc)-OH,
deprotect, couple with Fmoc-
Trp(Boc)-OH, deprotect
Trp(Boc)-Lys(Boc)-[Pro or Phe]-Asp(OtBu)-[Leu or Ala]-NH-PAL
STEP 6
1. Split and encode
2. Couple with Fmoc-Ile-OH
or Fmoc-Gly-OH
Trp(Boc)-Lys(Boc)-[Pro or Phe]-Asp(OtBu)-[Leu or Ala]-NH-PAL
[Ile or Gly]
STEP 7-10
1. Pool, deprotect, encode
2. Couple with Fmoc-Ser(OtBu)-OH,
Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH
-OH, and Fmoc-Met-OH with
appropriate deprotections
Trp(Boc)-Lys(Boc)-[Pro or Phe]-Asp(OtBu)-[Leu or Ala]-NH-PAL
[Ile or Gly]-Ser(OtBu)-Asp(OtBu)-Leu-Met
STEP 11
Deprotect and cleave from resin
Met-Leu-Asp-Ser-[Ile or Gly]-Trp-Lys-[Pro or Phe]-Asp-[Leu or Ala]-NH2

FIG. 10

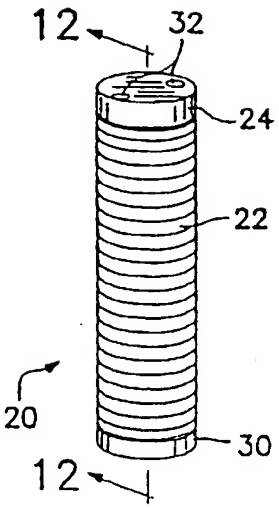


FIG. 11

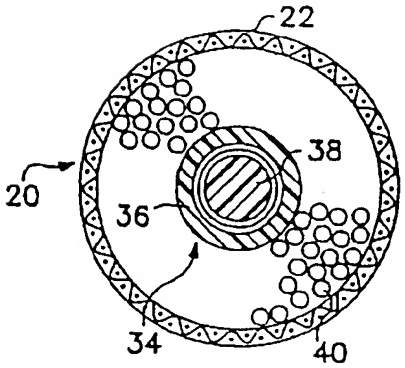


FIG. 13

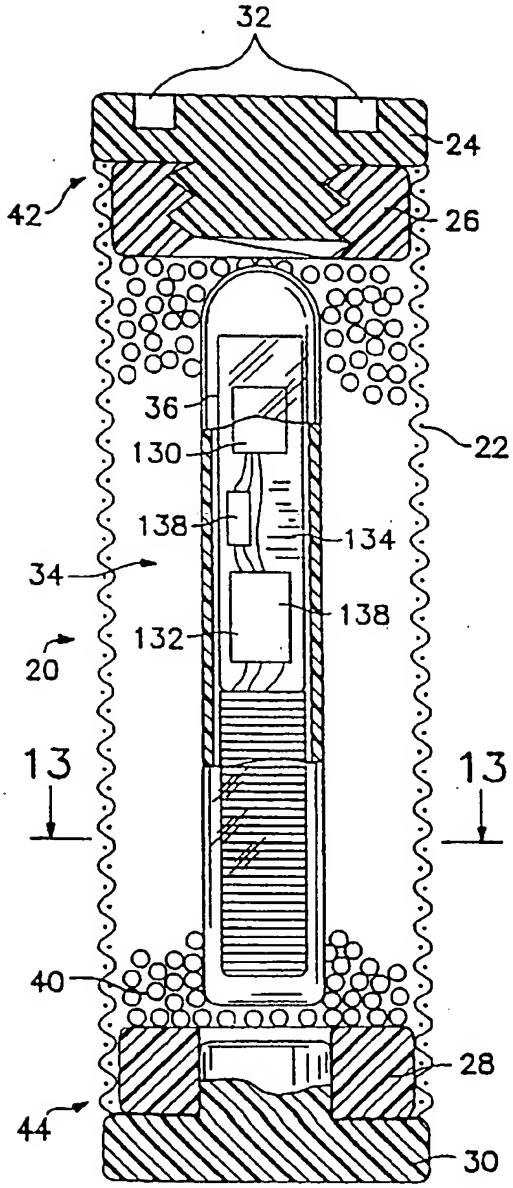


FIG. 12

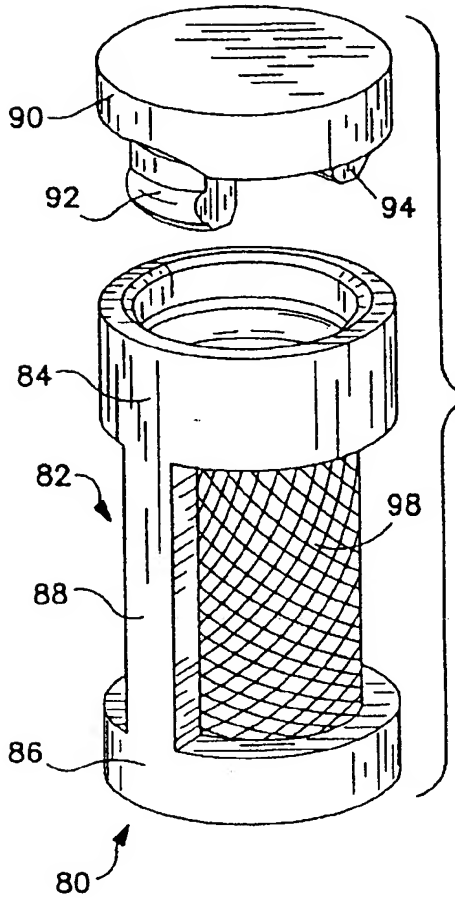


FIG. 14

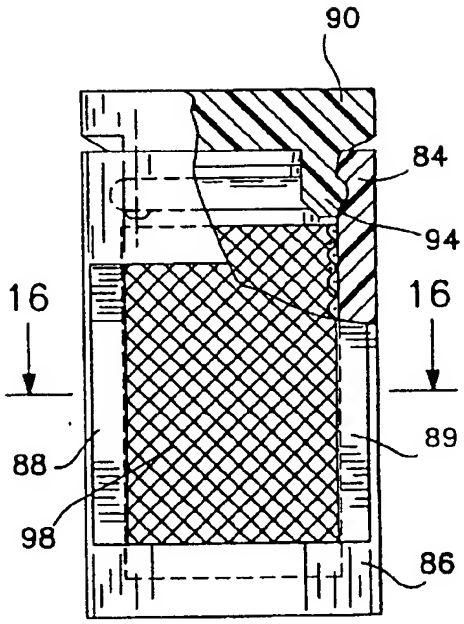


FIG. 15

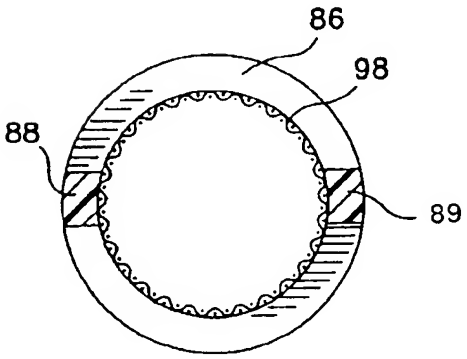


FIG. 16

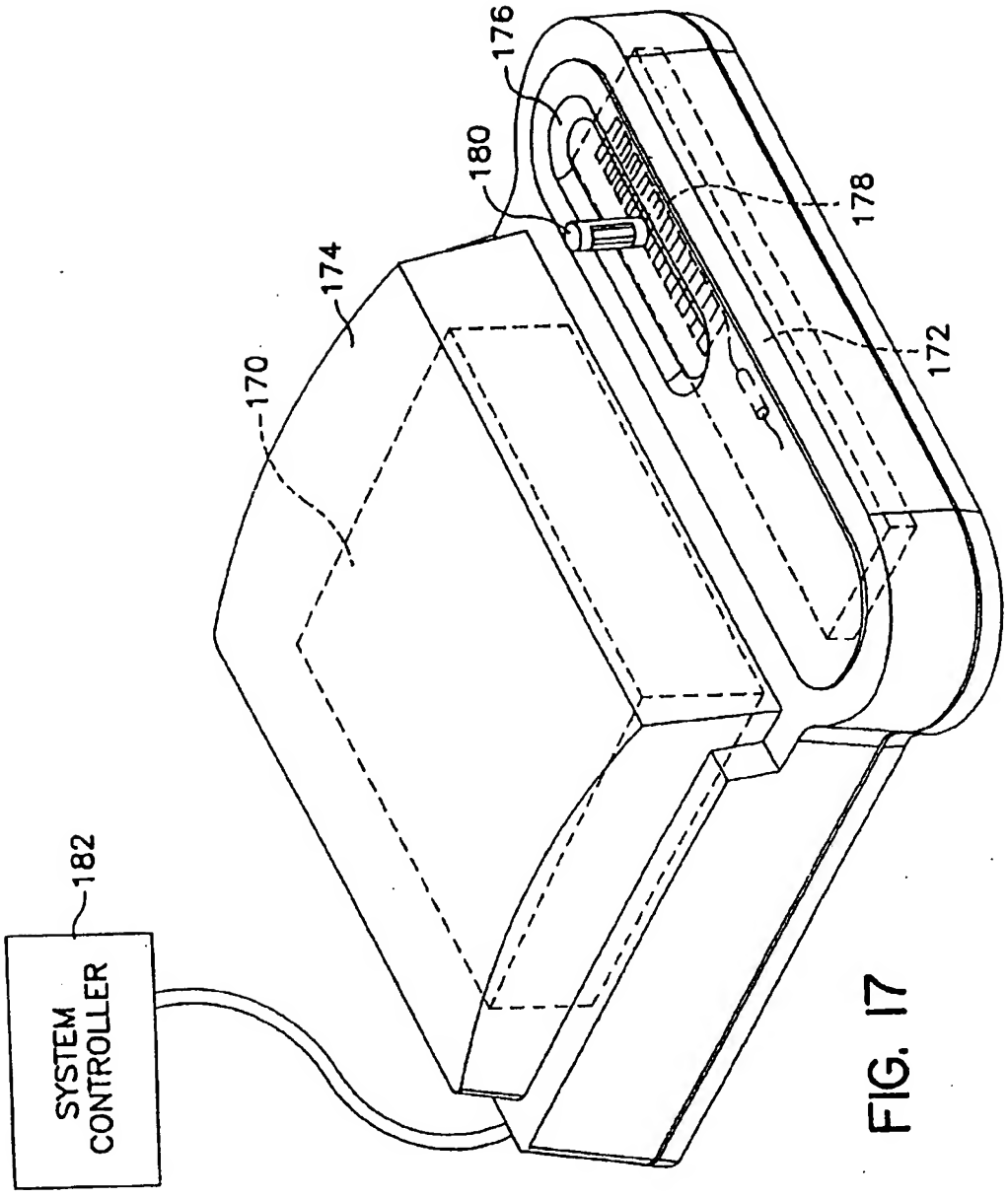


FIG. 17

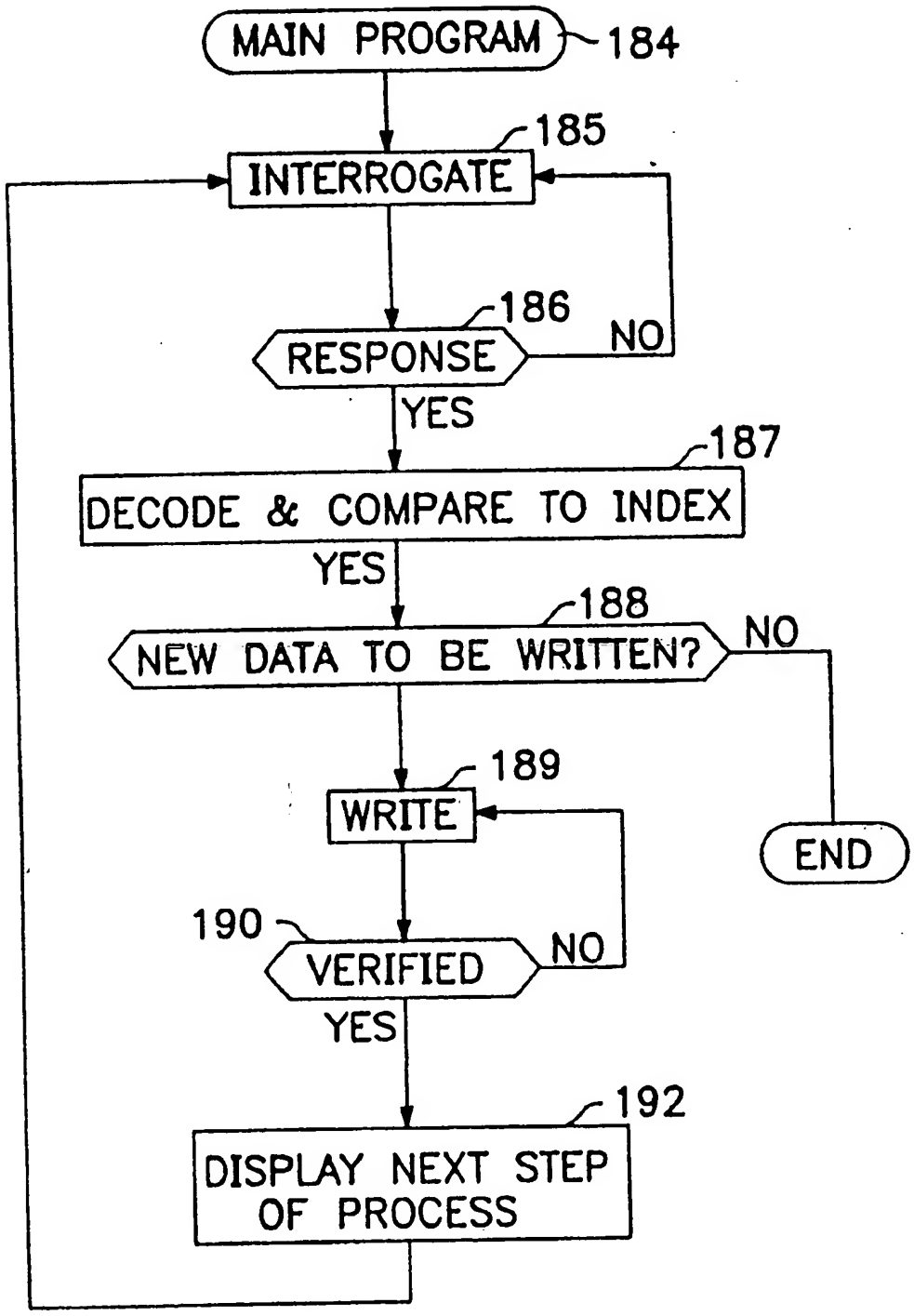

MAIN PROGRAM
184
185
INTERROGATE
186
RESPONSE
NO
YES
187
DECODE & COMPARE TO INDEX
YES
188
NEW DATA TO BE WRITTEN?
NO
189
WRITE
END
190
VERIFIED
NO
YES
192
DISPLAY NEXT STEP OF PROCESS

FIG. 18

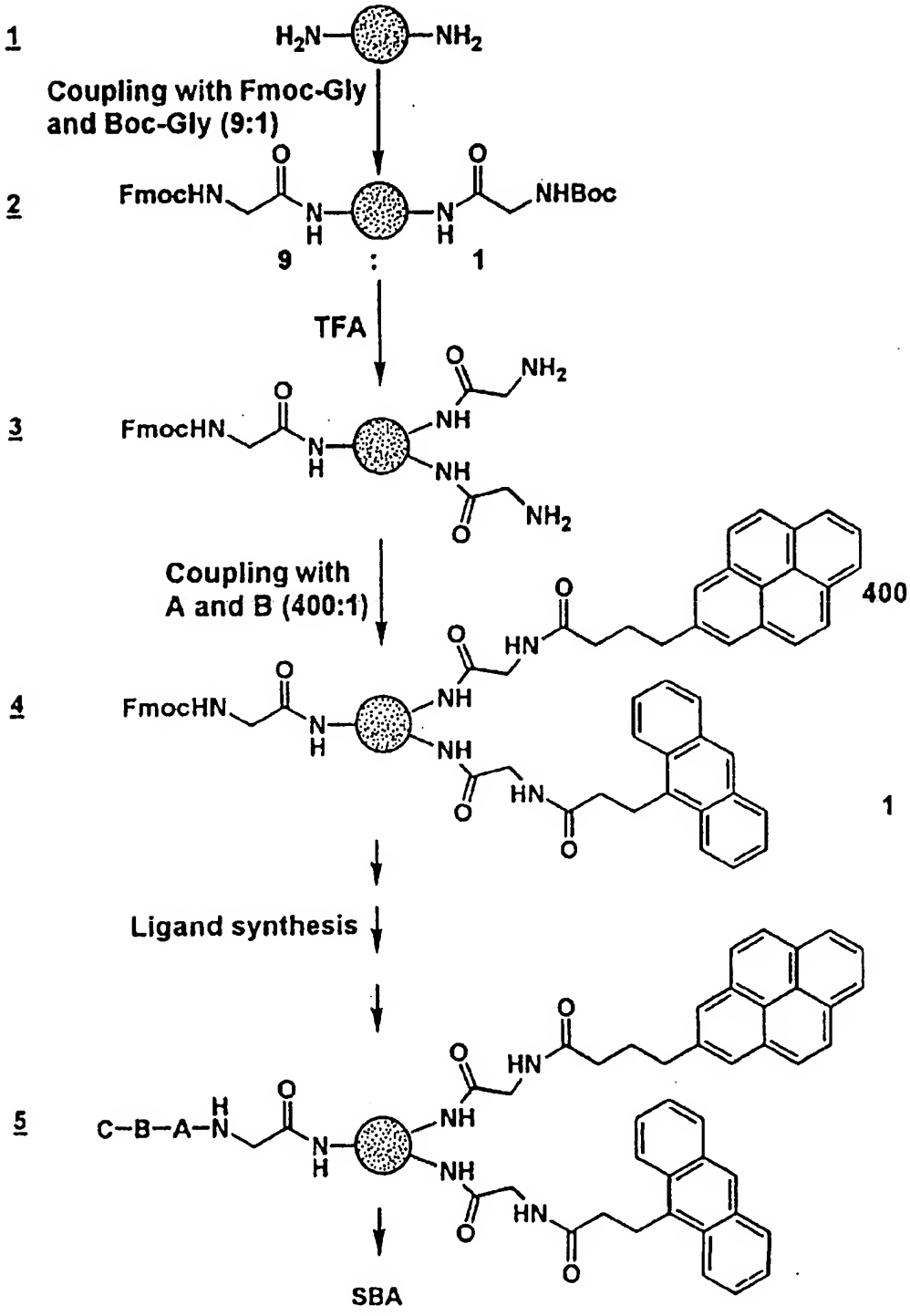

1
H_2N
NH_2
Coupling with Fmoc-Gly and Boc-Gly (9:1)
2
FmocHN
NHBoc
9 : 1
TFA
3
FmocHN
NH_2
Coupling with A and B (400:1)
400
4
FmocHN
1
Ligand synthesis
5
C–B–A–N
SBA

FIG. 19

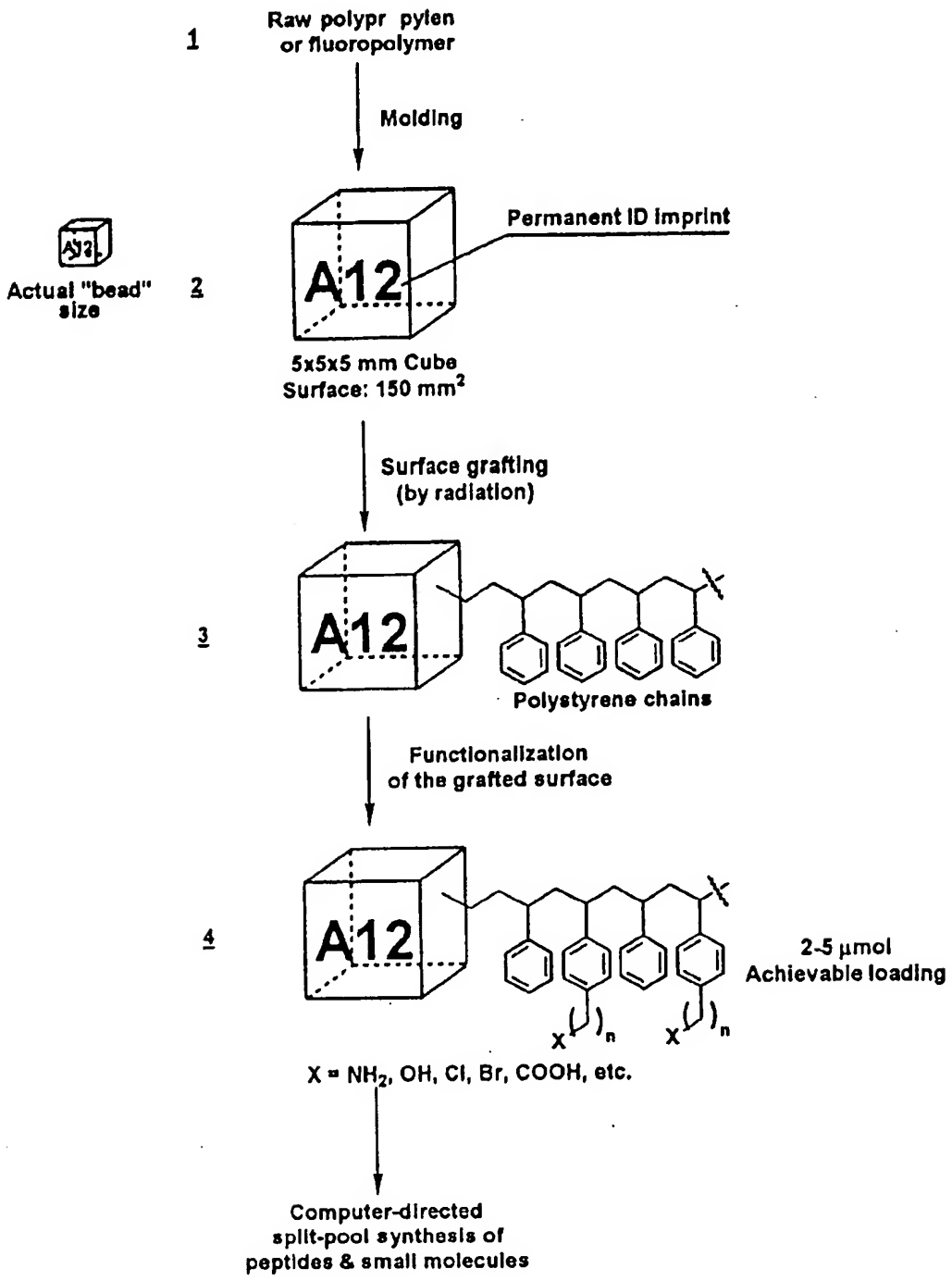

1
Raw polypr pylen or fluoropolymer
Molding
Actual "bead" size
2
A12
Permanent ID imprint
5x5x5 mm Cube
Surface: 150 mm2
Surface grafting (by radiation)
3
A12
Polystyrene chains
Functionalization of the grafted surface
4
A12
2-5 µmol Achievable loading
X = NH2, OH, Cl, Br, COOH, etc.
Computer-directed split-pool synthesis of peptides & small molecules

FIG. 20

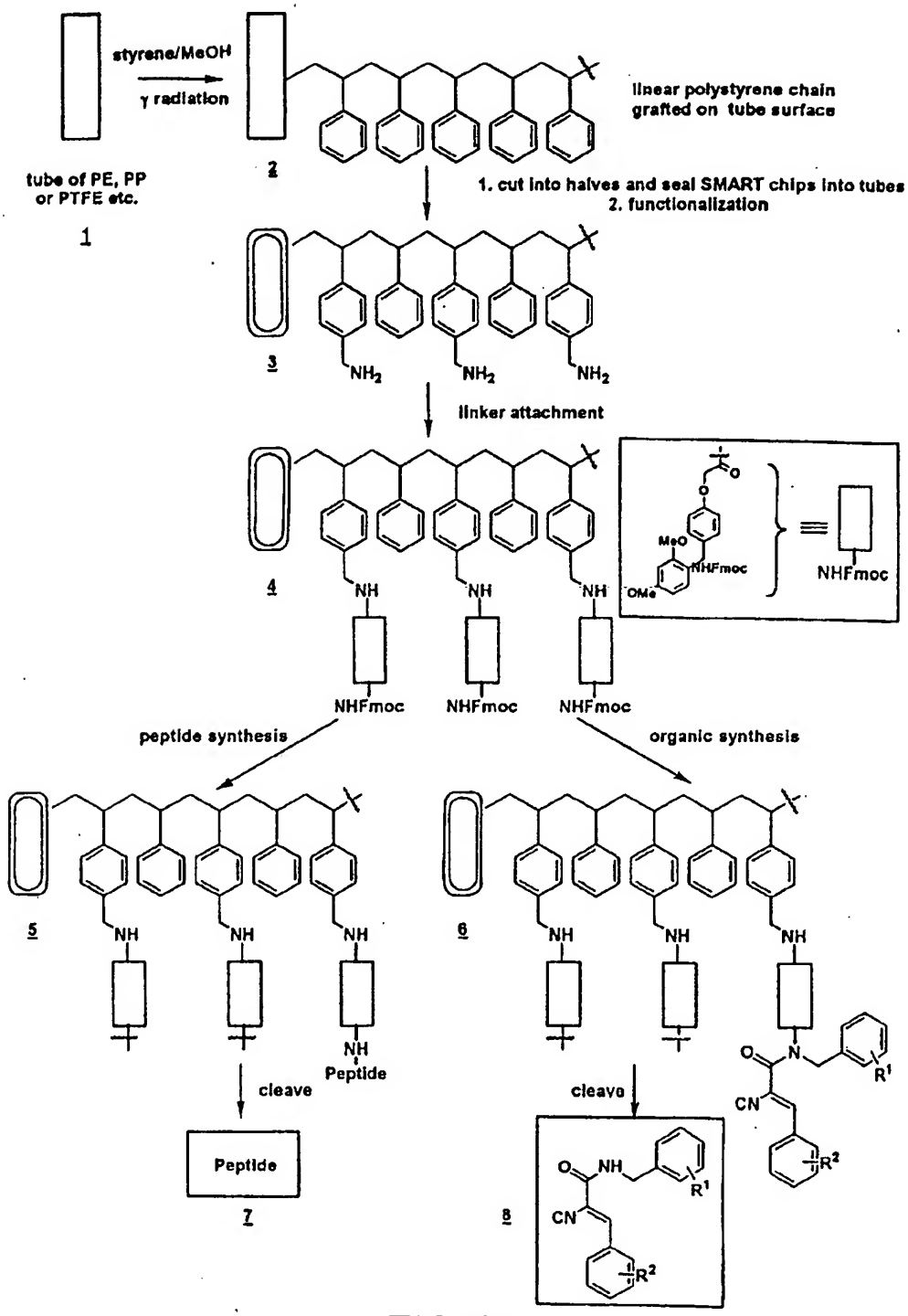

styrene/MeOH
γ radiation
linear polystyrene chain grafted on tube surface
tube of PE, PP or PTFE etc.
1
2
1. cut into halves and seal SMART chips into tubes
2. functionalization
3
NH2
linker attachment
4
NH
NHFmoc
MeO
OMe
peptide synthesis
organic synthesis
5
6
Peptide
cleave
7
8
CN
R1
R2

FIG. 21

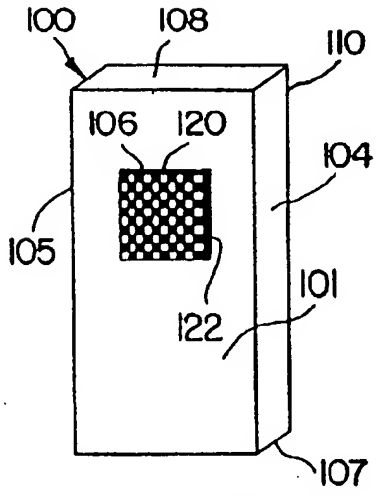


FIG. 22

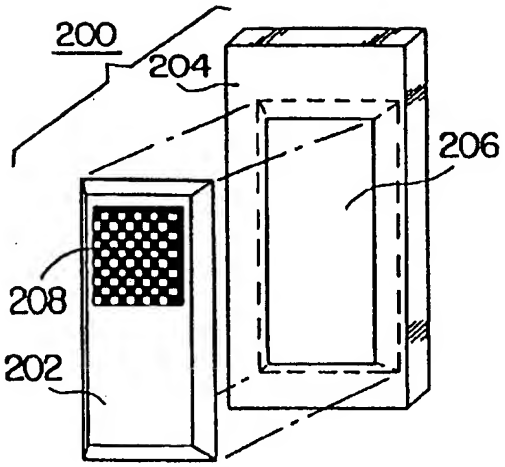


FIG. 23

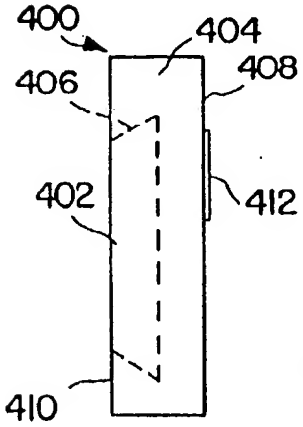


FIG. 25

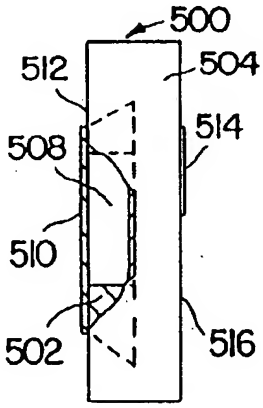


FIG. 26

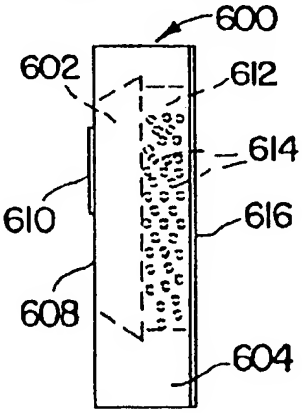


FIG. 27

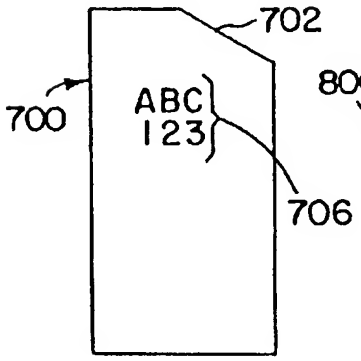


FIG. 28

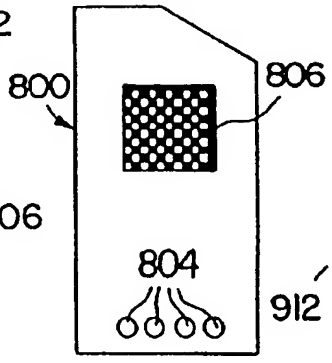


FIG. 29

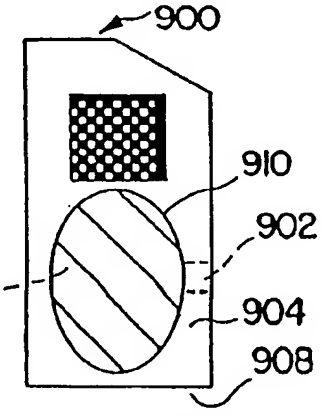


FIG. 30

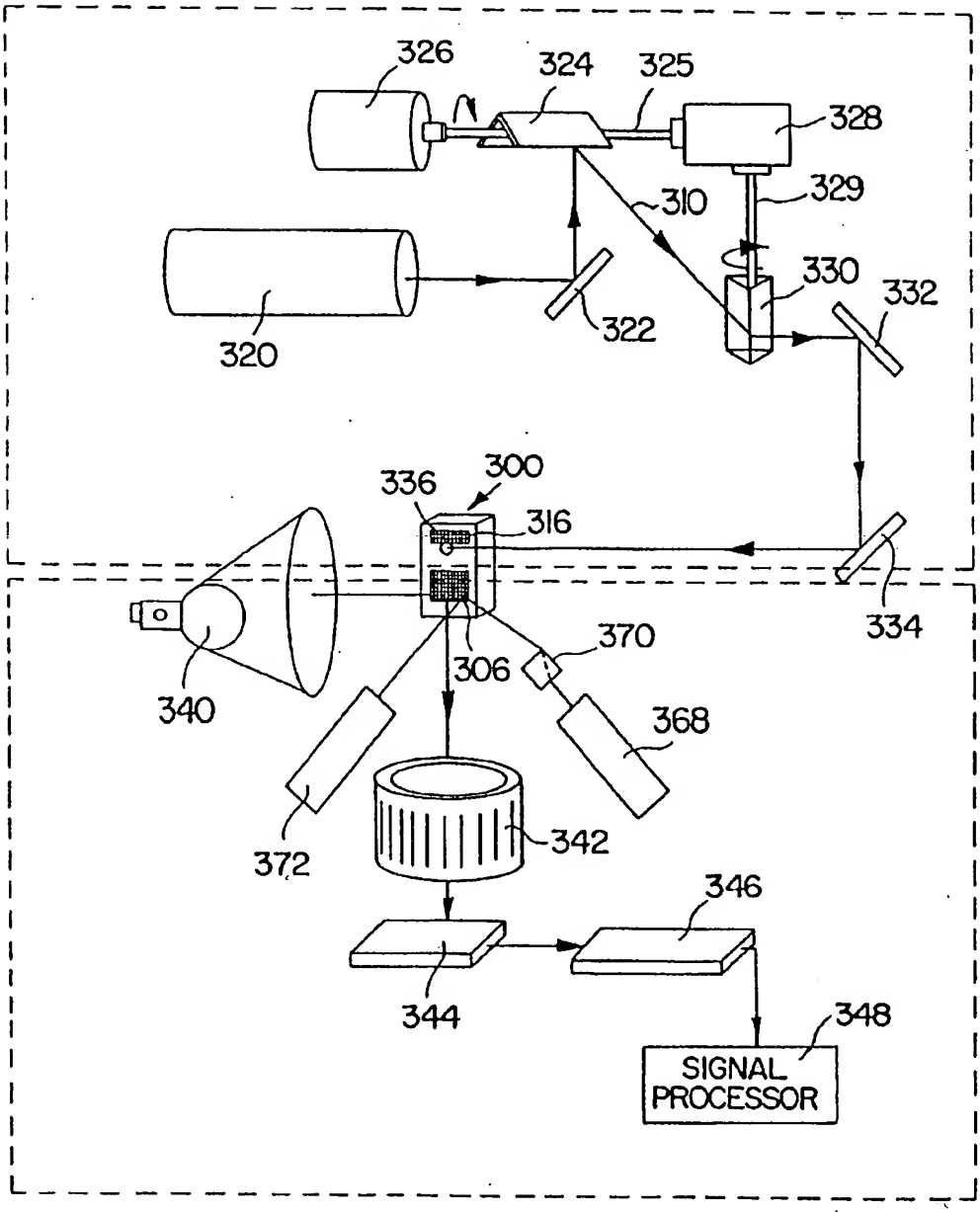

326
324
325
328
329
310
330
332
320
322
336
300
316
334
370
306
340
368
342
372
346
344
348
SIGNAL PROCESSOR

FIG. 24

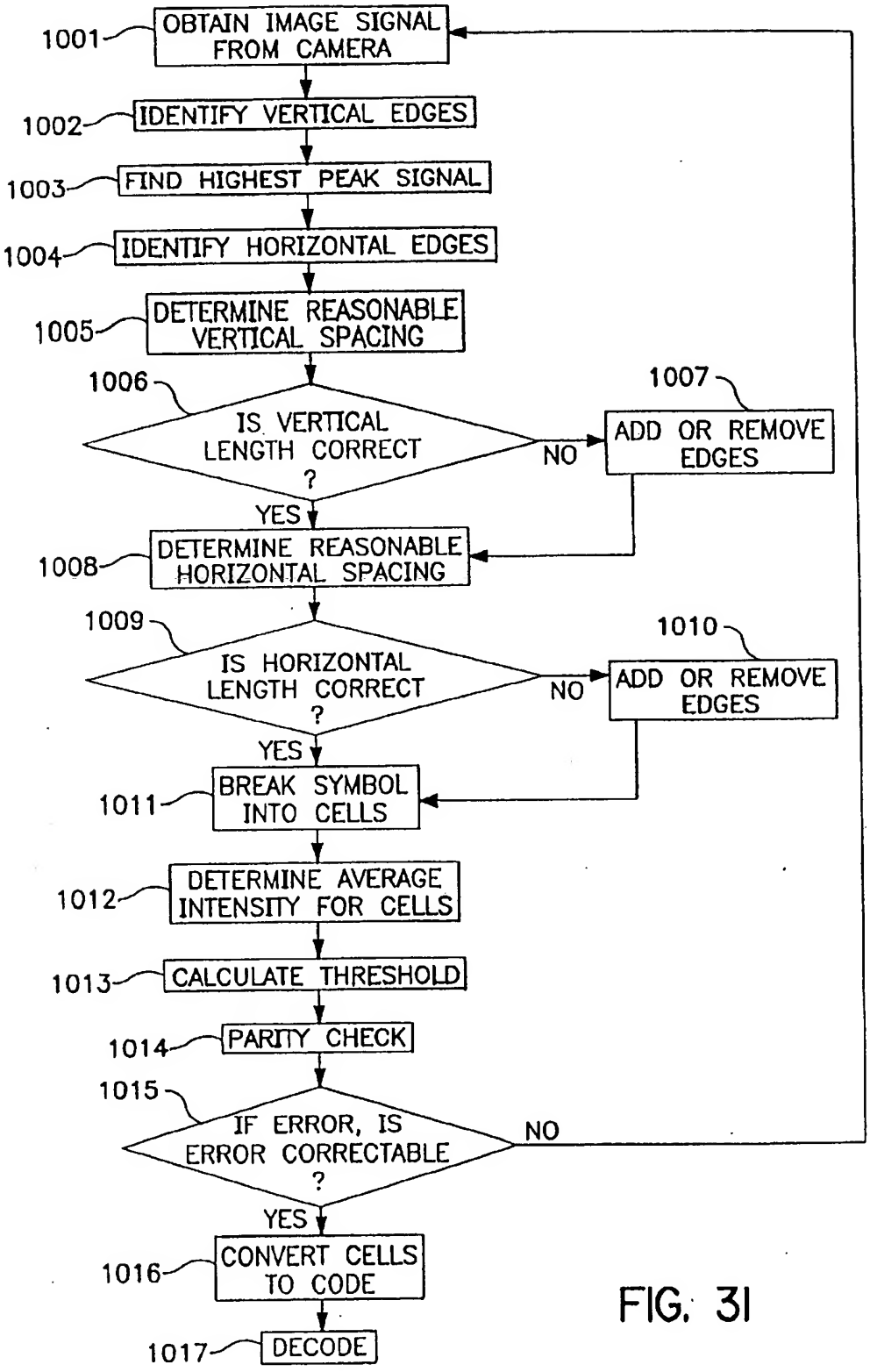

1001
OBTAIN IMAGE SIGNAL FROM CAMERA
1002
IDENTIFY VERTICAL EDGES
1003
FIND HIGHEST PEAK SIGNAL
1004
IDENTIFY HORIZONTAL EDGES
1005
DETERMINE REASONABLE VERTICAL SPACING
1006
IS VERTICAL LENGTH CORRECT ?
NO
1007
ADD OR REMOVE EDGES
YES
1008
DETERMINE REASONABLE HORIZONTAL SPACING
1009
IS HORIZONTAL LENGTH CORRECT ?
NO
1010
ADD OR REMOVE EDGES
YES
1011
BREAK SYMBOL INTO CELLS
1012
DETERMINE AVERAGE INTENSITY FOR CELLS
1013
CALCULATE THRESHOLD
1014
PARITY CHECK
1015
IF ERROR, IS ERROR CORRECTABLE ?
NO
YES
1016
CONVERT CELLS TO CODE
1017
DECODE

FIG. 31

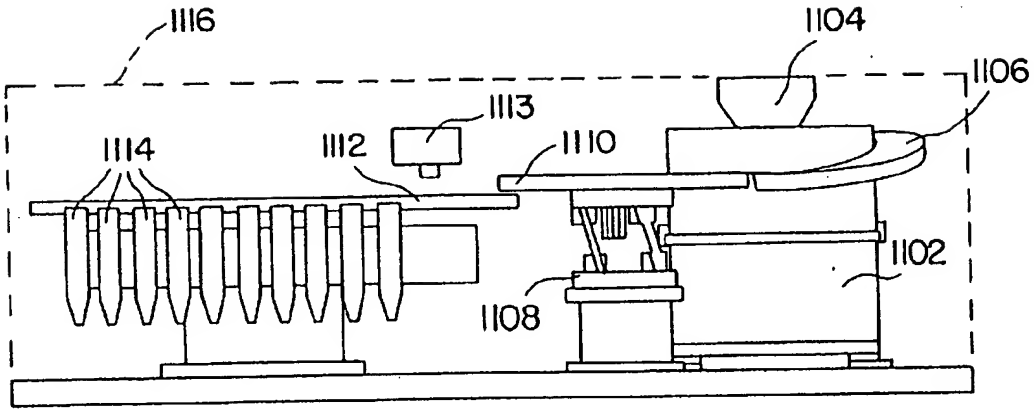


FIG. 32

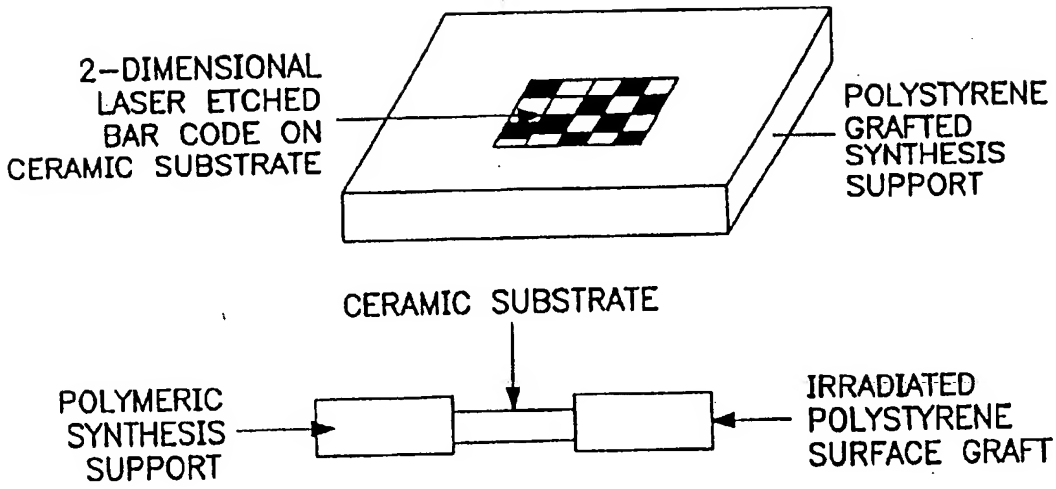


FIG. 33A

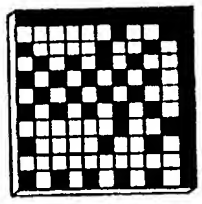


FIG. 33B

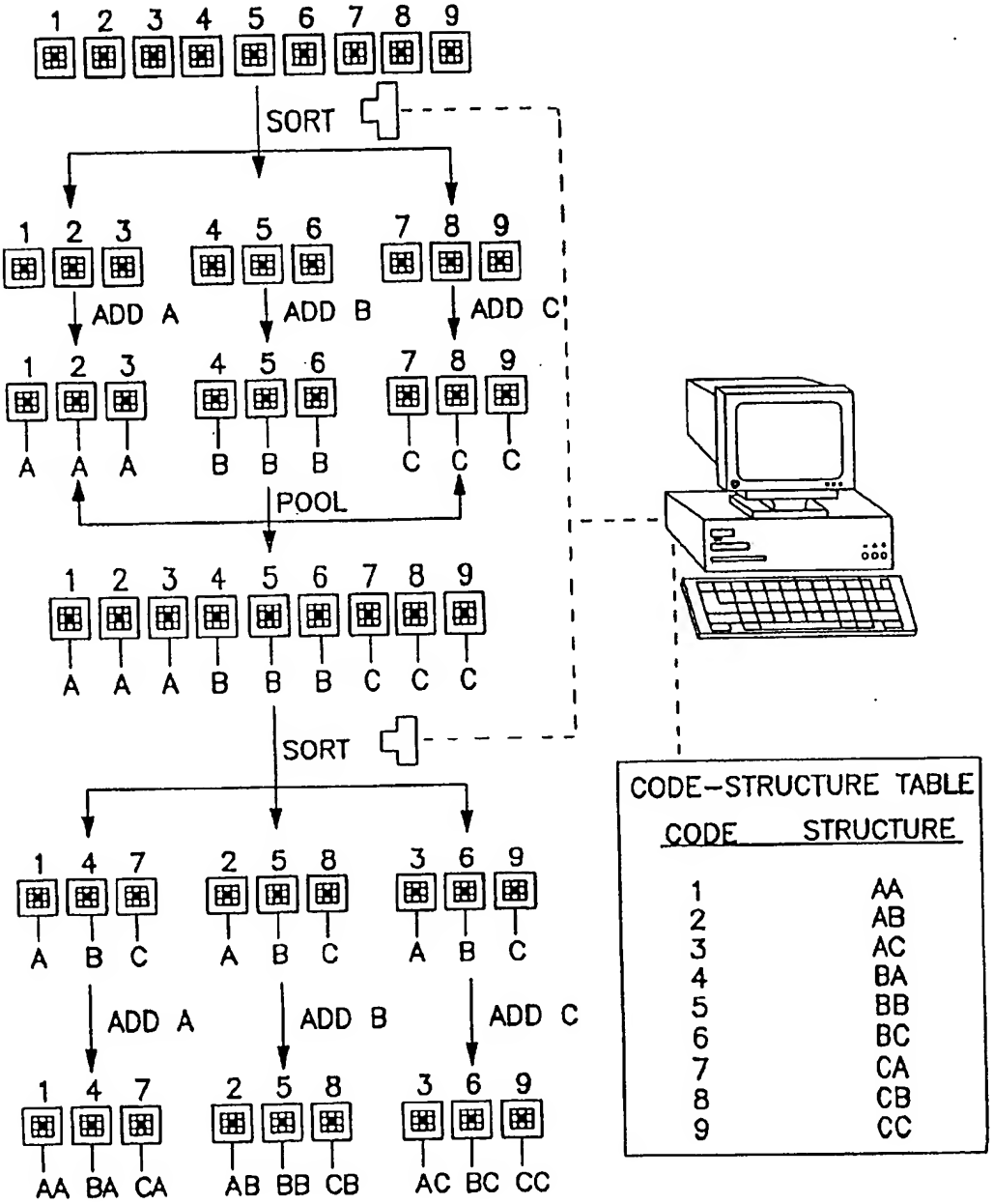

1 2 3 4 5 6 7 8 9
SORT
1 2 3 4 5 6 7 8 9
ADD A
ADD B
ADD C
1 2 3 4 5 6 7 8 9
A A A B B B C C C
POOL
1 2 3 4 5 6 7 8 9
A A A B B B C C C
SORT
1 4 7 2 5 8 3 6 9
A B C A B C A B C
ADD A
ADD B
ADD C
1 4 7 2 5 8 3 6 9
AA BA CA AB BB CB AC BC CC
CODE-STRUCTURE TABLE
CODE STRUCTURE
1 AA
2 AB
3 AC
4 BA
5 BB
6 BC
7 CA
8 CB
9 CC

FIG. 33C

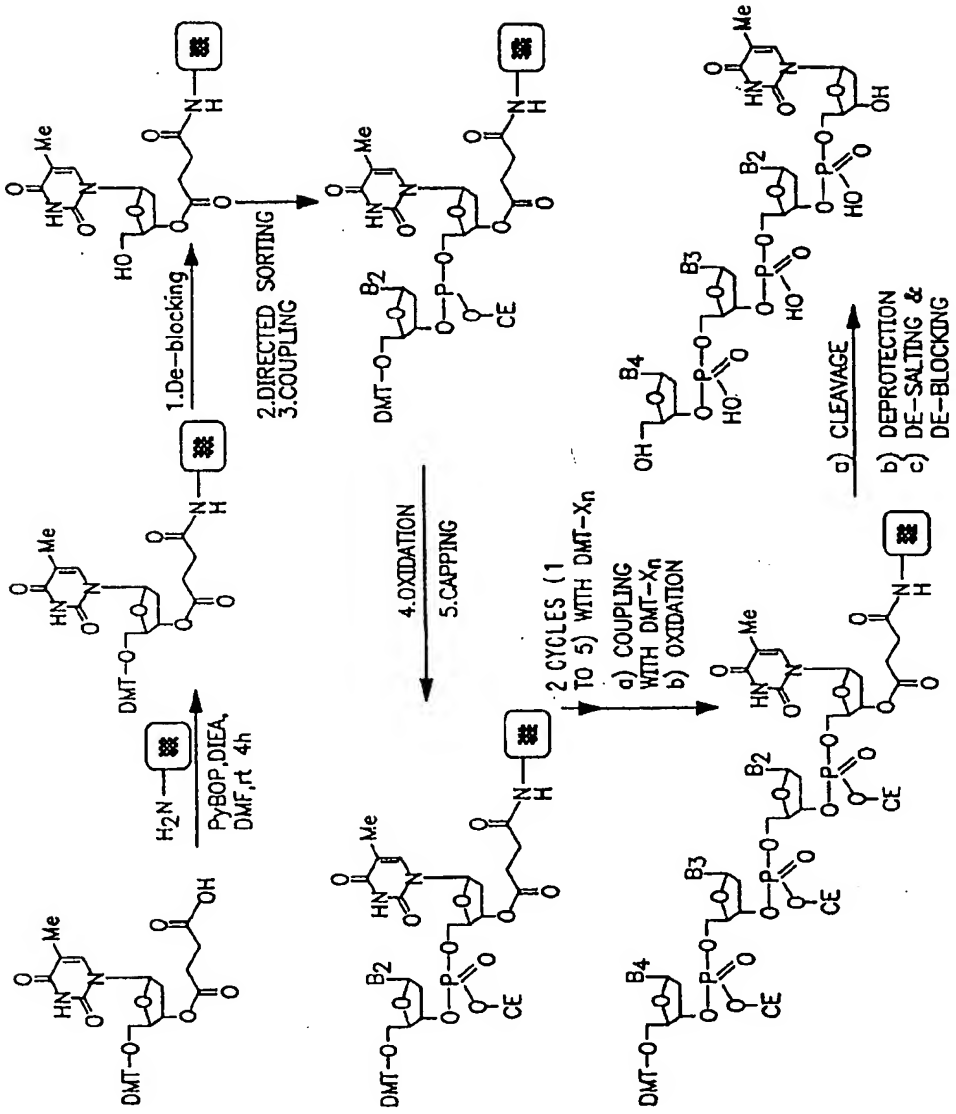

PyBOP,DIEA,
DMF,rt 4h
1.De-blocking
2.DIRECTED SORTING
3.COUPLING
4.OXIDATION
5.CAPPING
2 CYCLES (1 TO 5) WITH DMT-Xn
a) COUPLING WITH DMT-Xn
b) OXIDATION
a) CLEAVAGE
b) DEPROTECTION
c) DE-SALTING & DE-BLOCKING

FIG. 33D

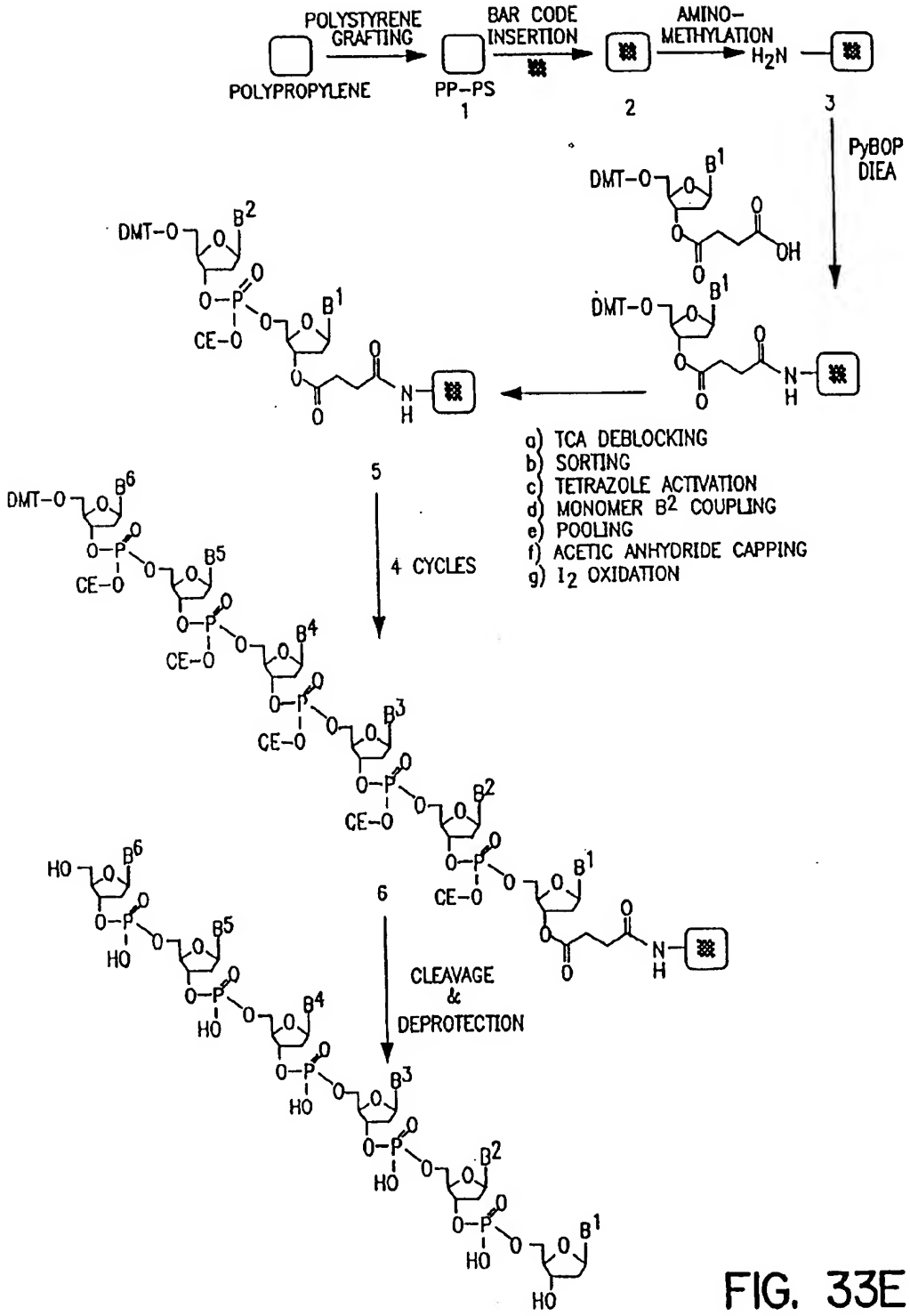

POLYSTYRENE GRAFTING
POLYPROPYLENE
PP-PS
1
BAR CODE INSERTION
2
AMINO-METHYLATION
H_2N
3
PyBOP DIEA
DMT-O
B^1
OH
B^2
CE-O
N H
a) TCA DEBLOCKING
b) SORTING
c) TETRAZOLE ACTIVATION
d) MONOMER B^2 COUPLING
e) POOLING
f) ACETIC ANHYDRIDE CAPPING
g) I_2 OXIDATION
5
4 CYCLES
B^3
B^4
B^5
B^6
HO
6
CLEAVAGE & DEPROTECTION

FIG. 33E

SCHEME 1

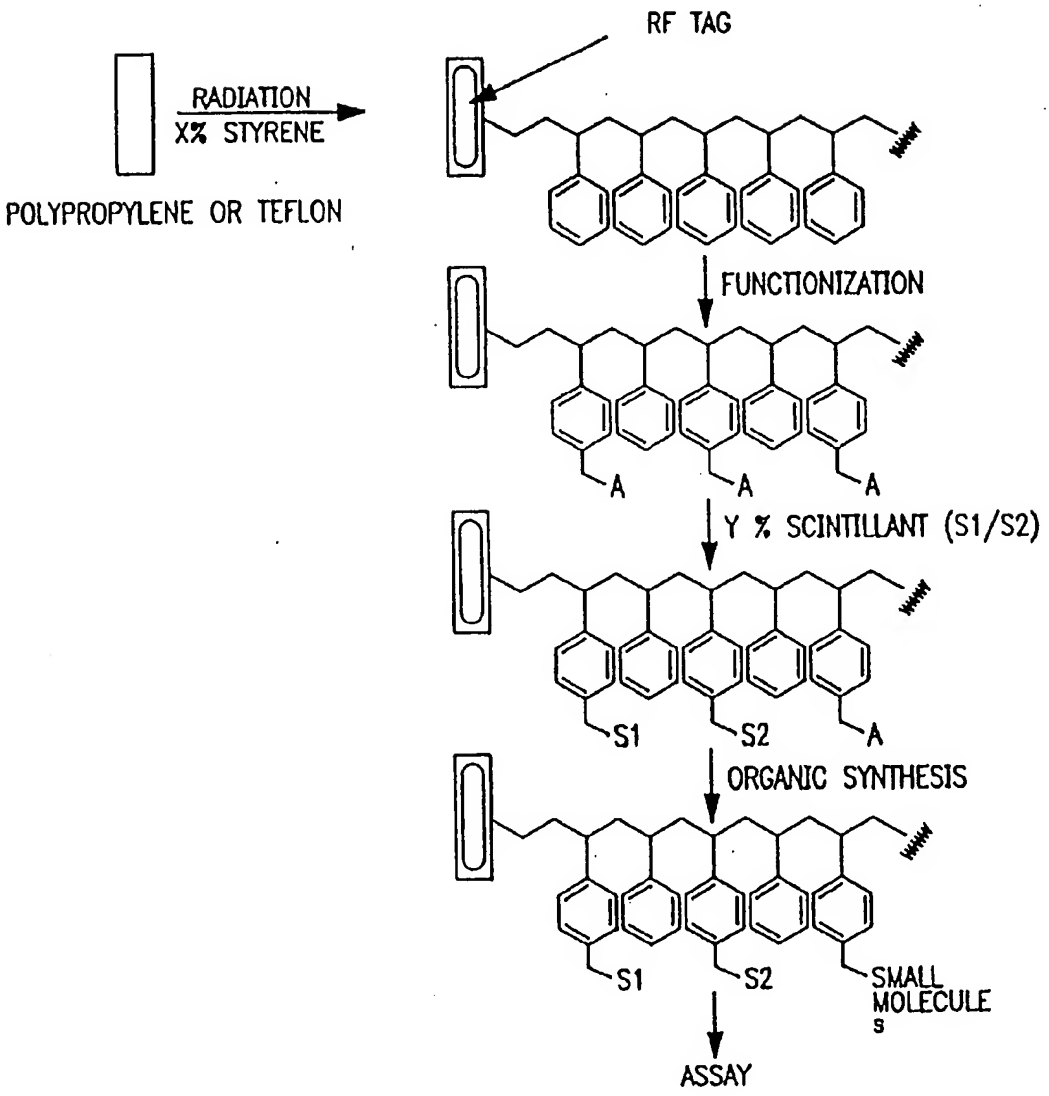


FIG. 34A

SCHEME 2

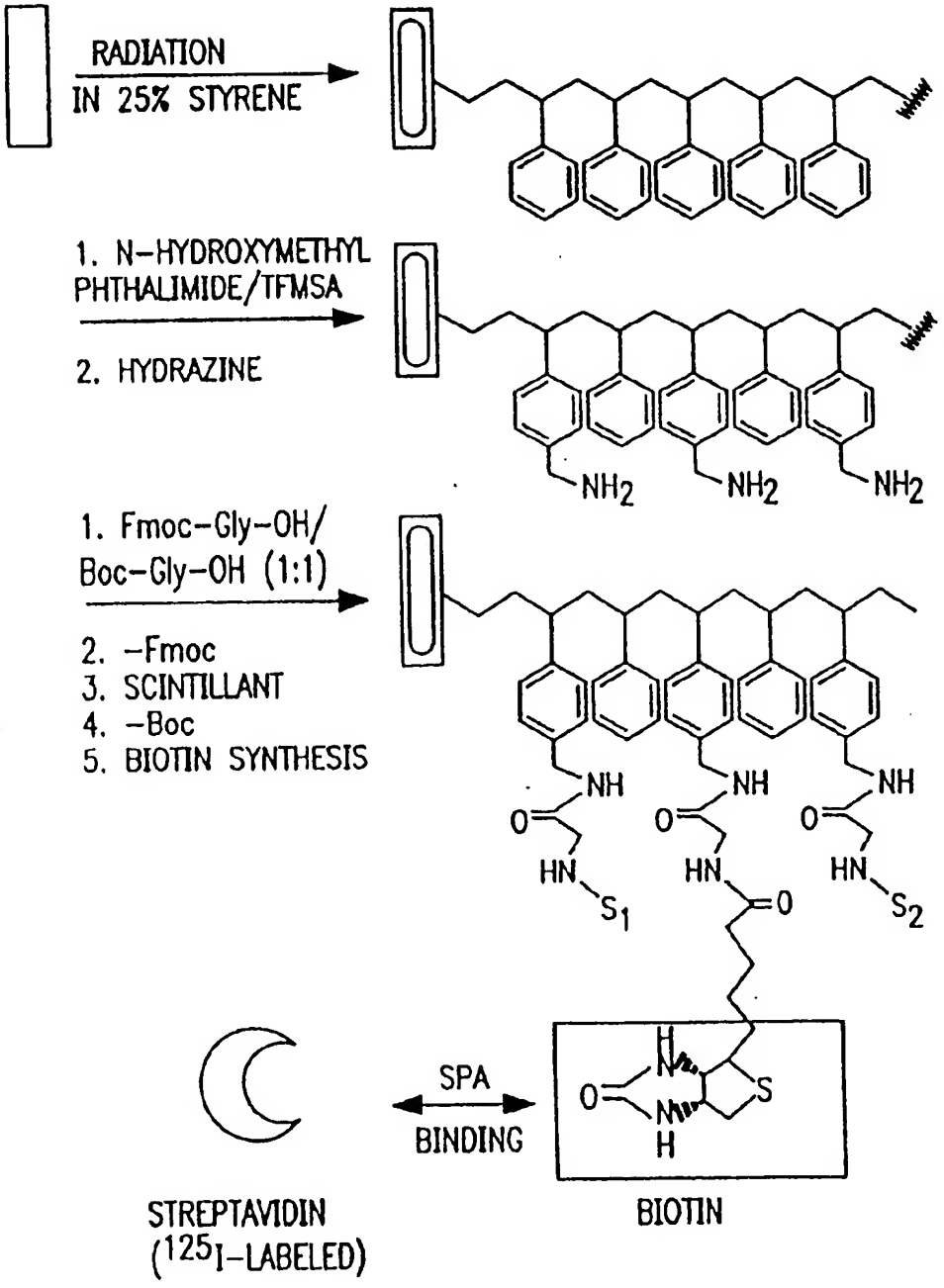


FIG. 34B

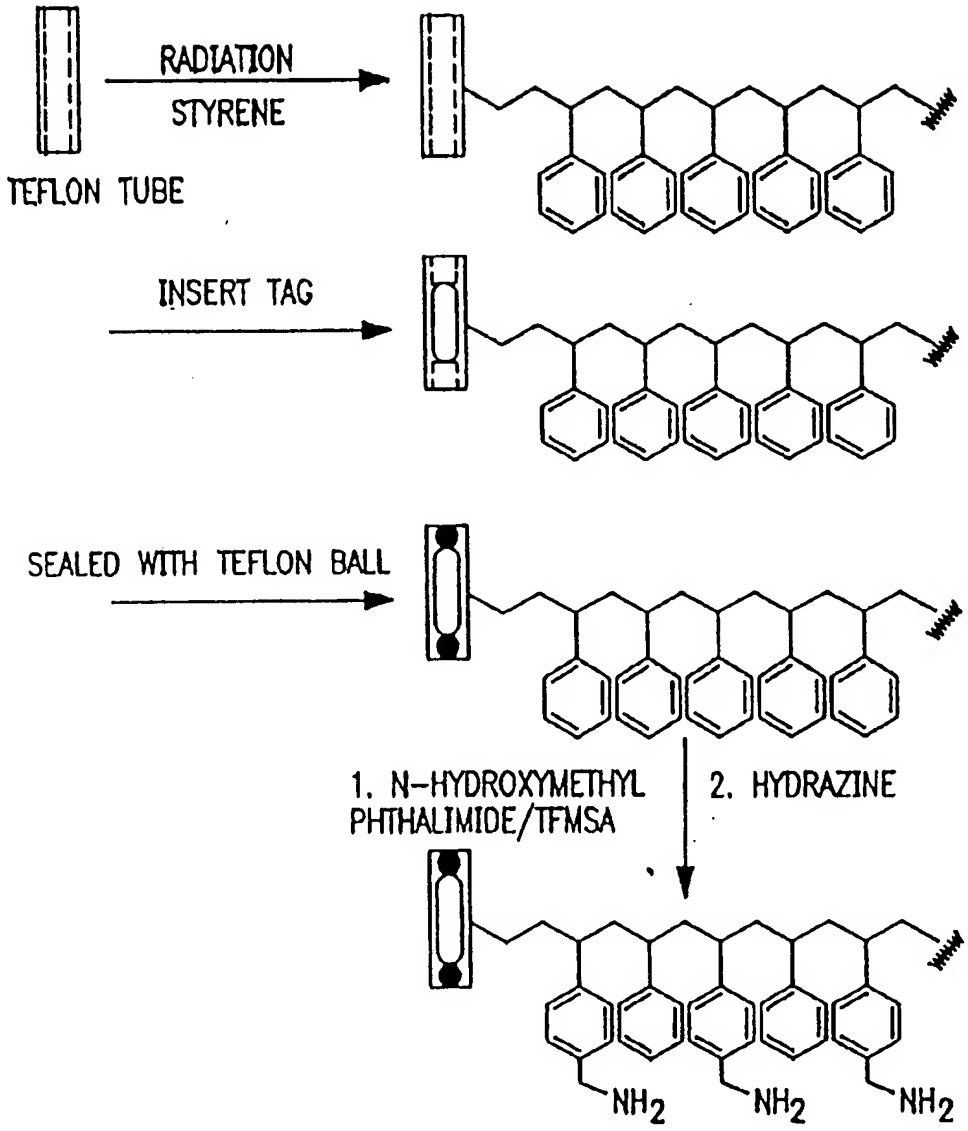

RADIATION
STYRENE
TEFLON TUBE
INSERT TAG
SEALED WITH TEFLON BALL
1. N-HYDROXYMETHYL PHTHALIMIDE/TFMSA
2. HYDRAZINE
NH2
NH2
NH2

FIG. 34C

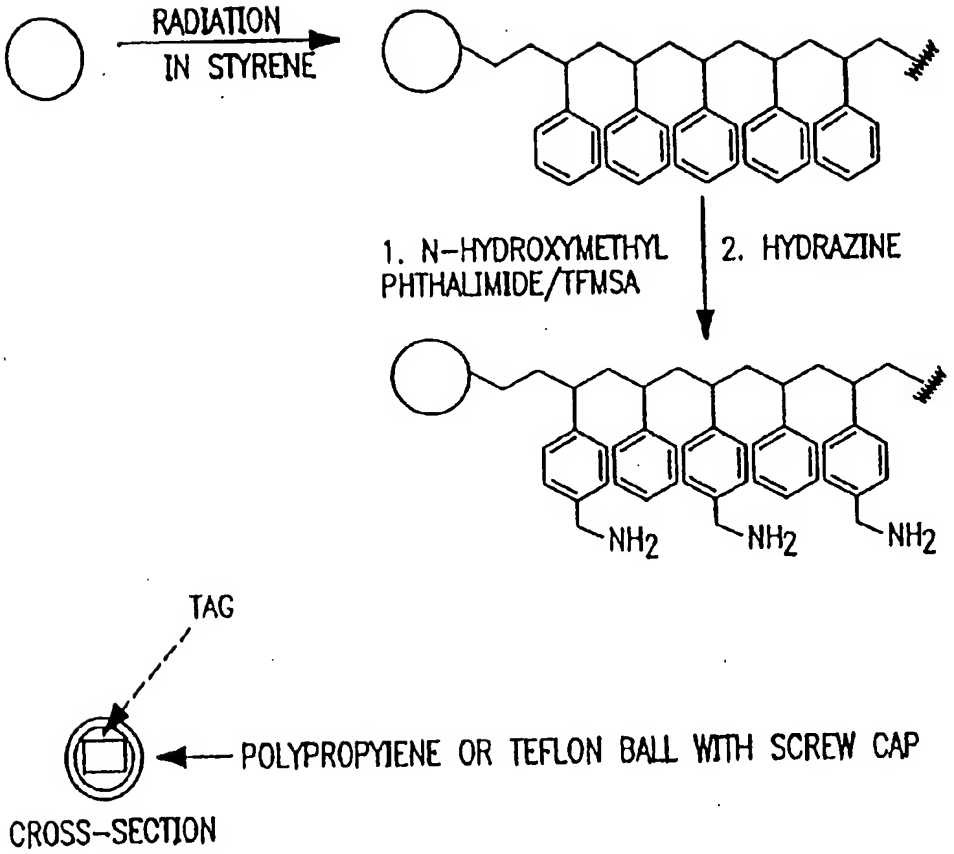

RADIATION
IN STYRENE
1. N-HYDROXYMETHYL PHTHALIMIDE/TFMSA
2. HYDRAZINE
NH2
NH2
NH2
TAG
POLYPROPYIENE OR TEFLON BALL WITH SCREW CAP
CROSS-SECTION

FIG. 34D

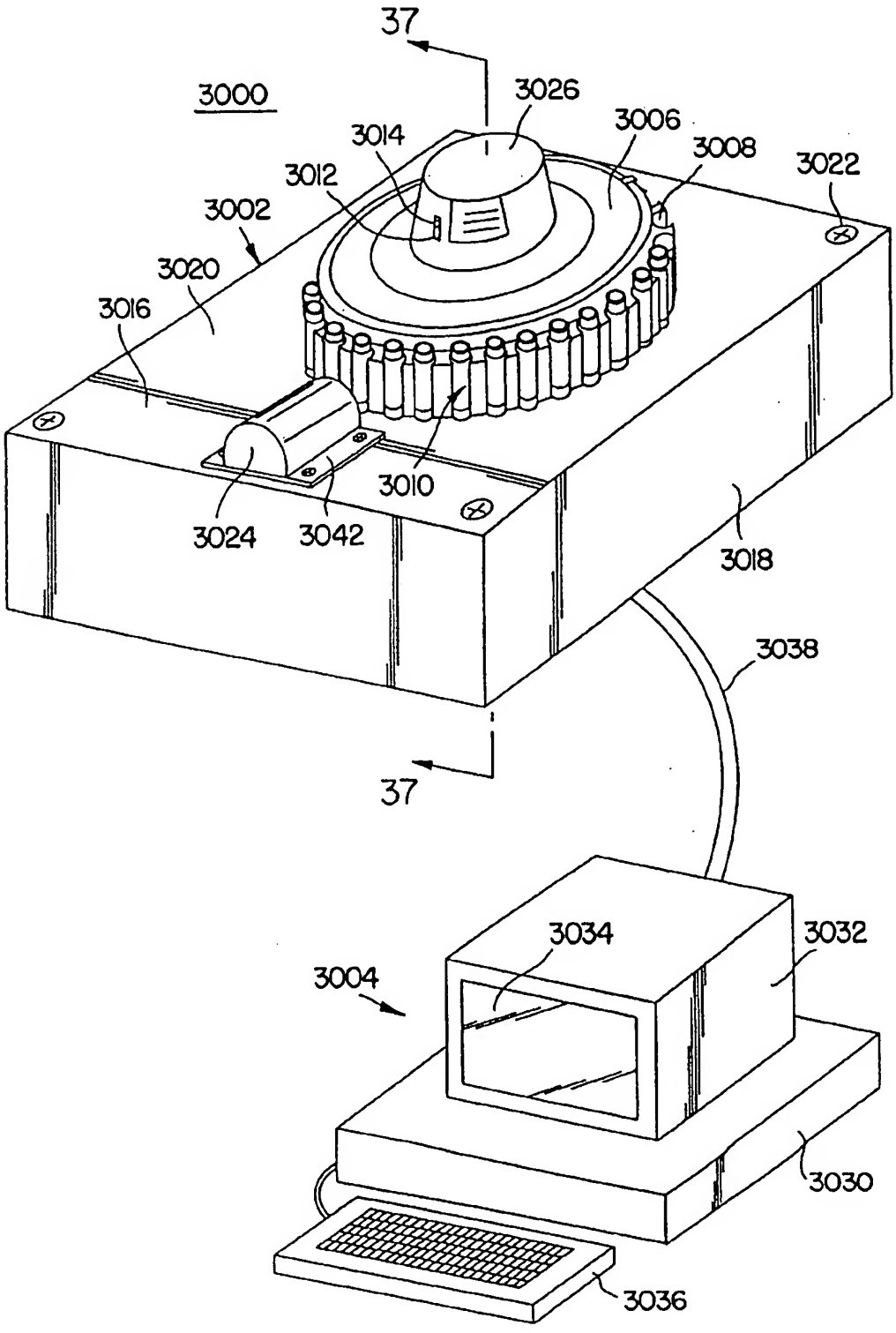

37
3000
3026
3006
3014
3008
3022
3012
3002
3020
3016
3010
3024
3042
3018
3038
37
3034
3032
3004
3030
3036

FIG. 35

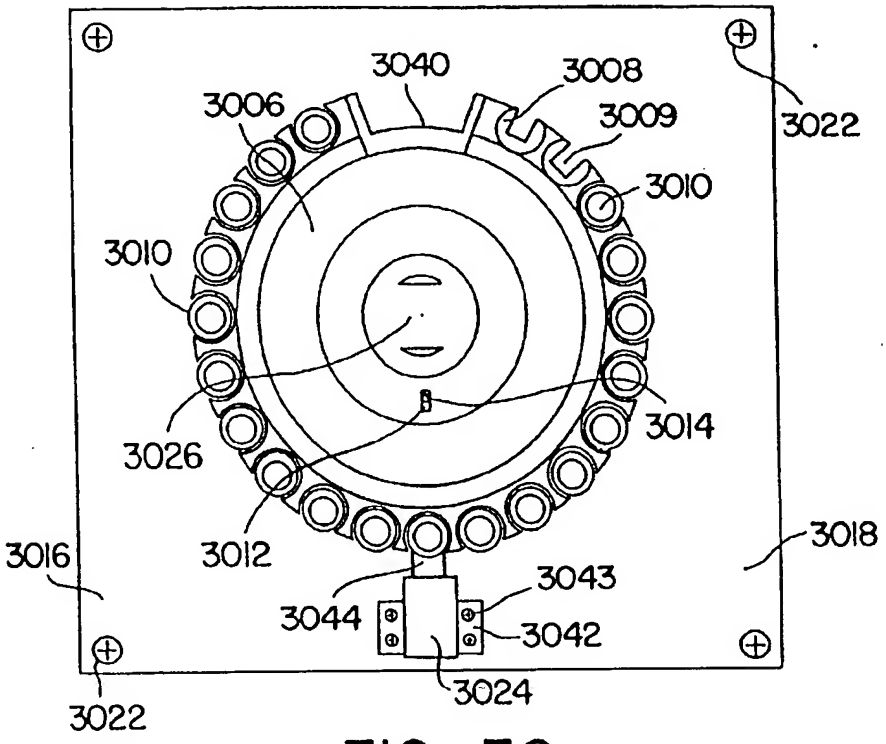

3040
3008
3006
3009
3022
3010
3010
3014
3026
3012
3018
3016
3043
3044
3042
3024
3022

FIG. 36

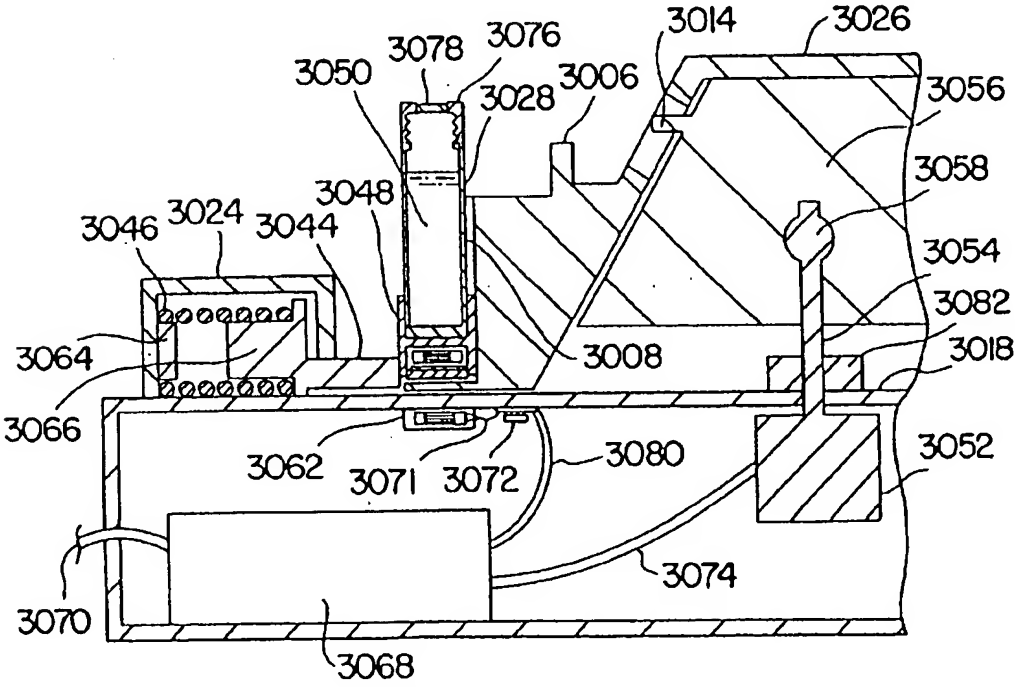

3014
3026
3078
3076
3050
3028
3006
3056
3058
3048
3024
3046
3044
3054
3082
3064
3008
3018
3066
3062
3071
3072
3080
3052
3074
3070
3068

FIG. 37

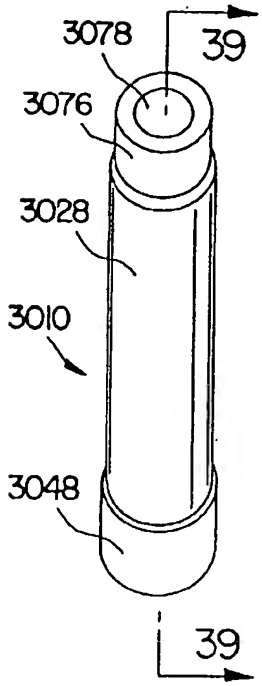


FIG. 38

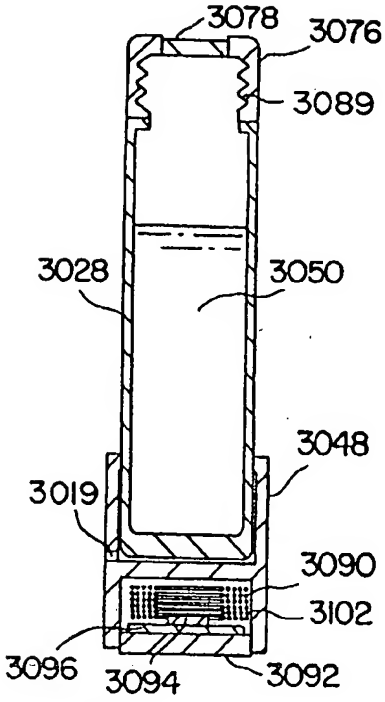


FIG. 39

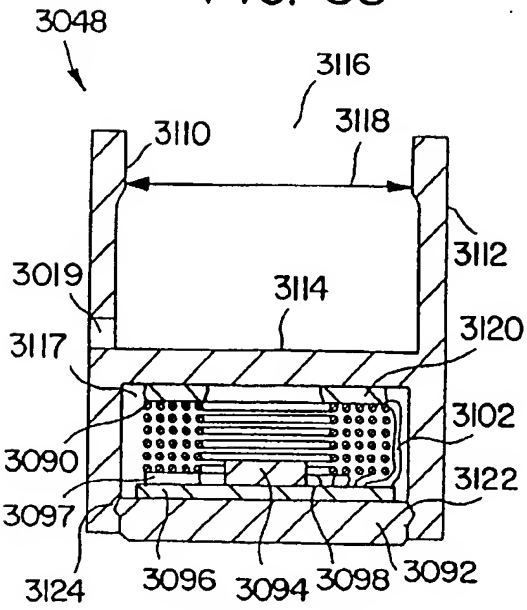


FIG. 40

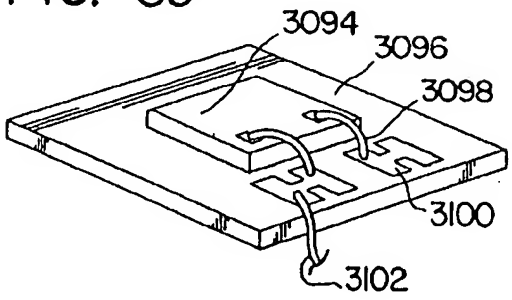


FIG. 41

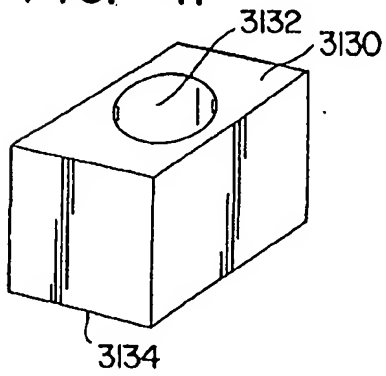


FIG. 42

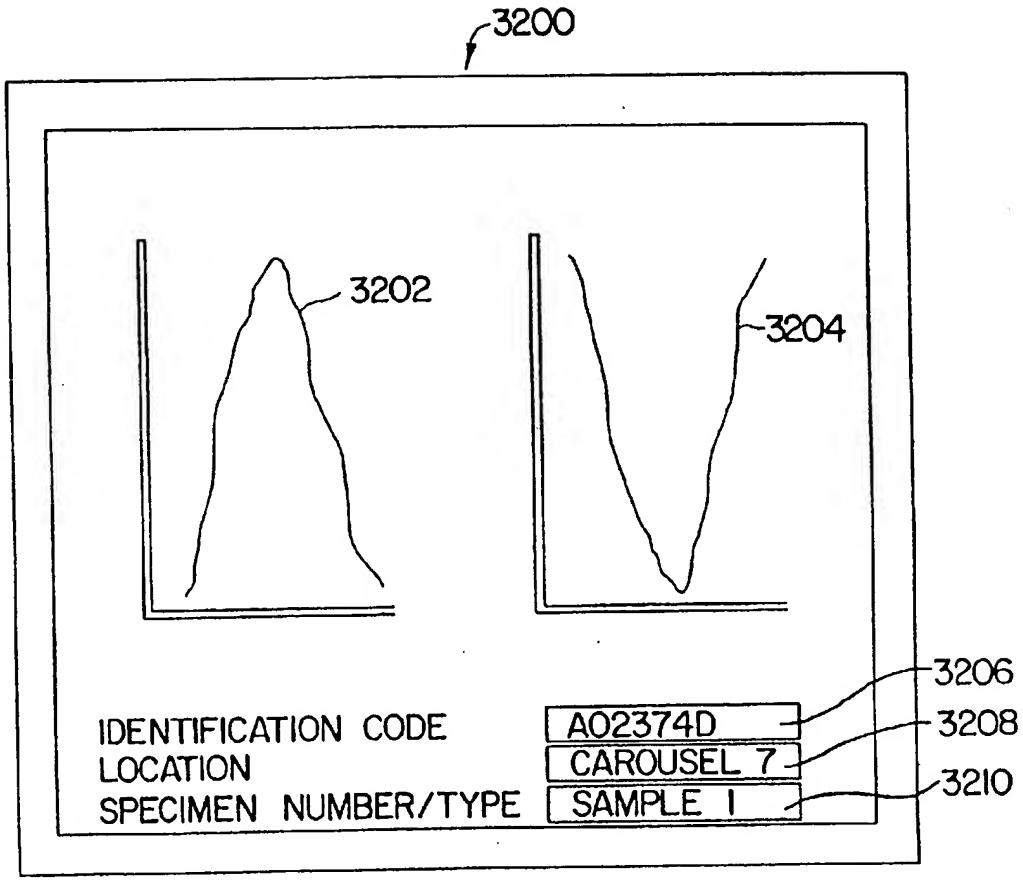

3200
3202
3204
3206
3208
3210
IDENTIFICATION CODE
LOCATION
SPECIMEN NUMBER/TYPE
A02374D
CAROUSEL 7
SAMPLE 1

FIG. 43

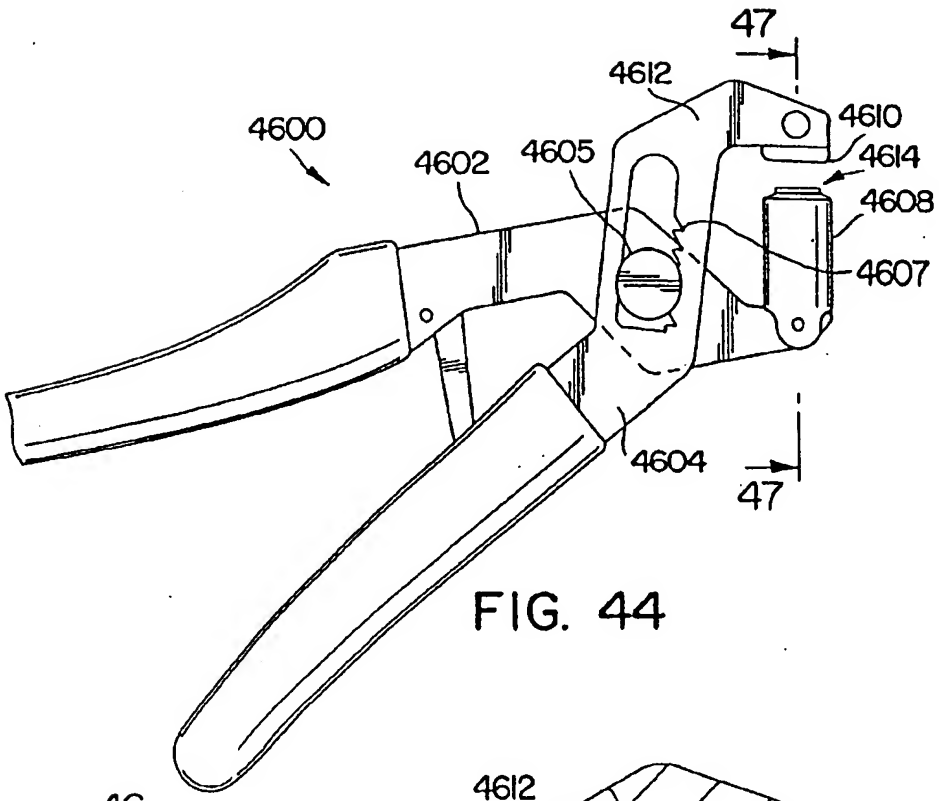


FIG. 44

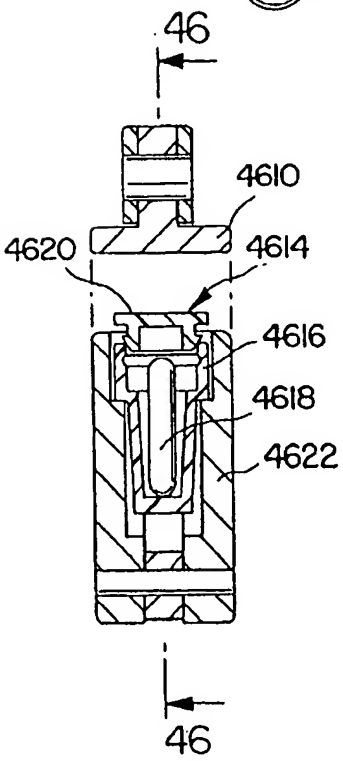


FIG. 45

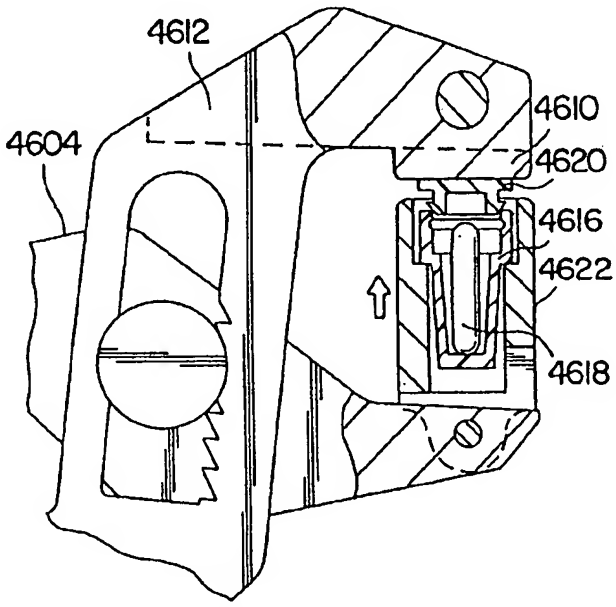


FIG. 46

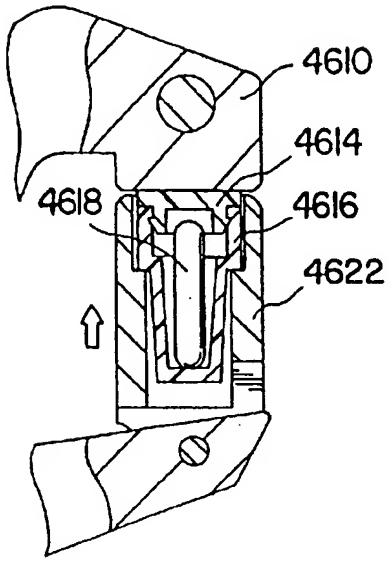


FIG. 47

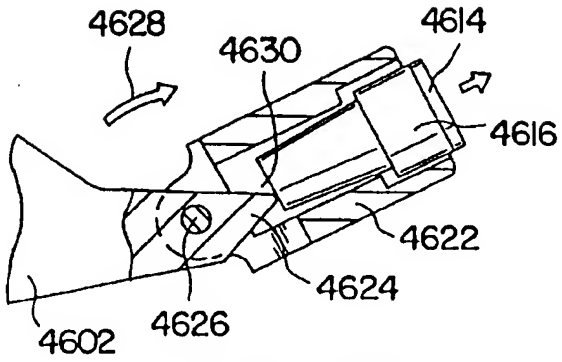


FIG. 48

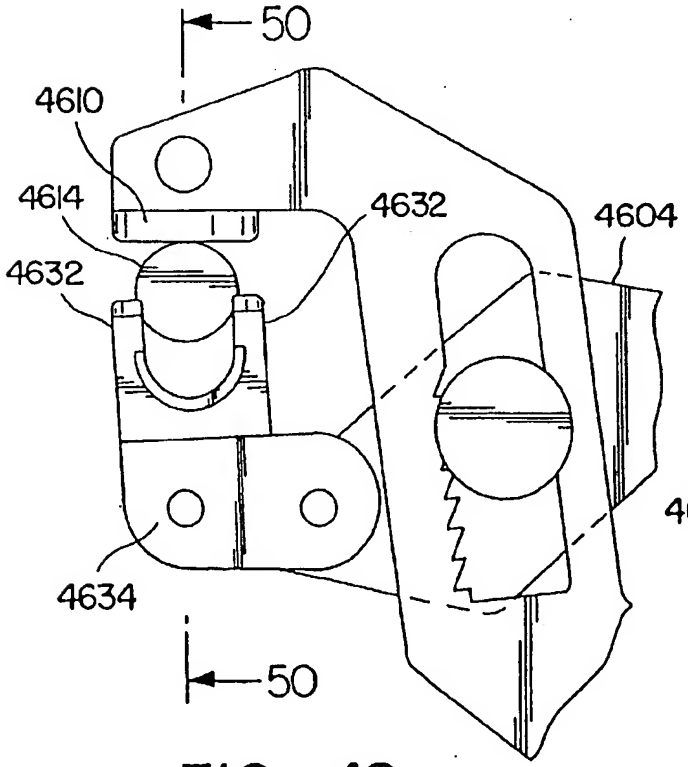


FIG. 49

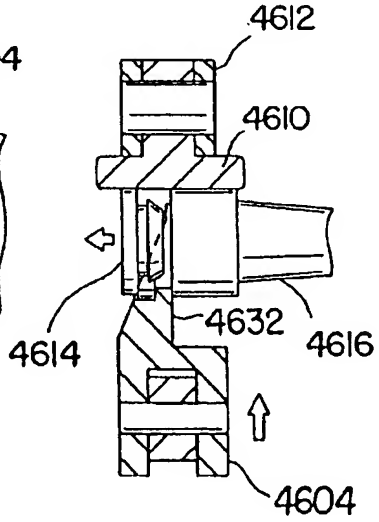


FIG. 50

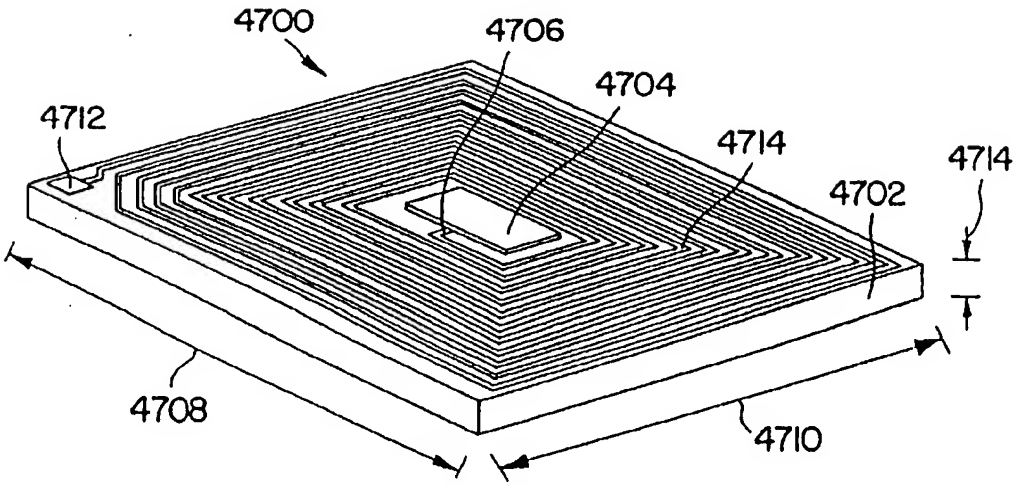


FIG. 51

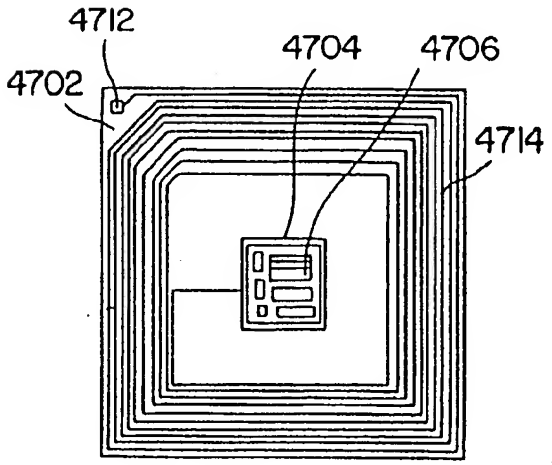


FIG. 52

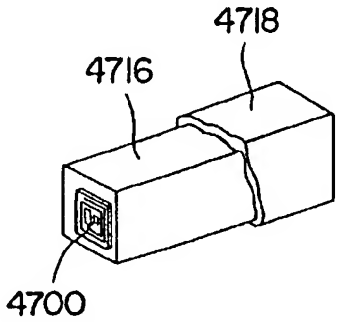


FIG. 53

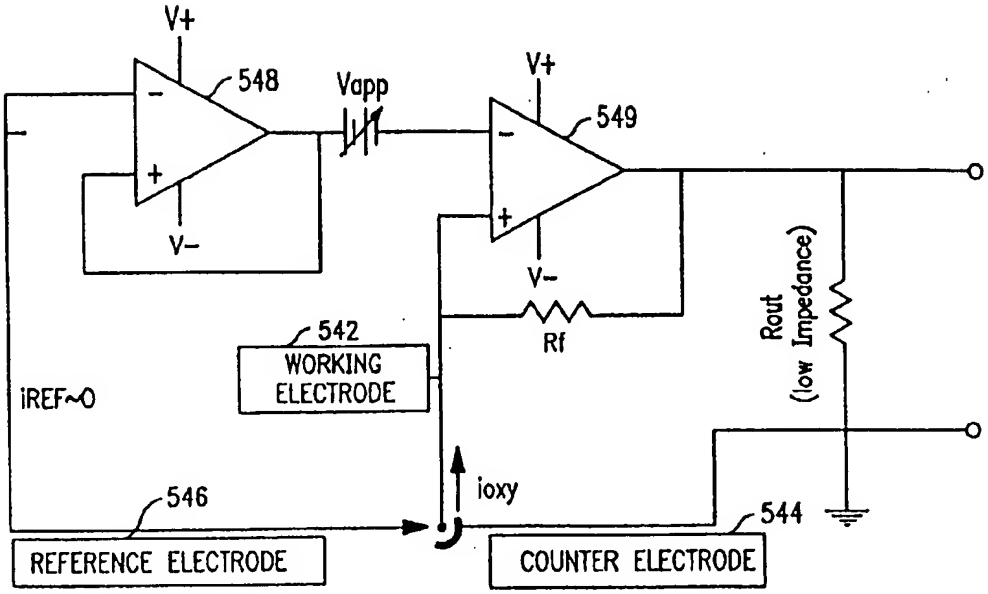

V+
548
Vapp
V+
549
V-
V-
Rf
542
WORKING ELECTRODE
iREF~0
Rout (low Impedance)
ioxy
546
544
REFERENCE ELECTRODE
COUNTER ELECTRODE

FIG. 54

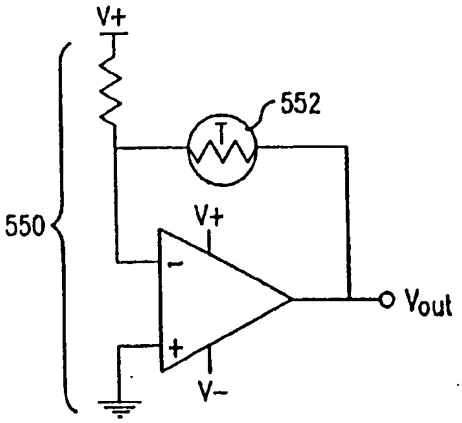

V+
552
T
550
V+
Vout
V-

FIG. 55

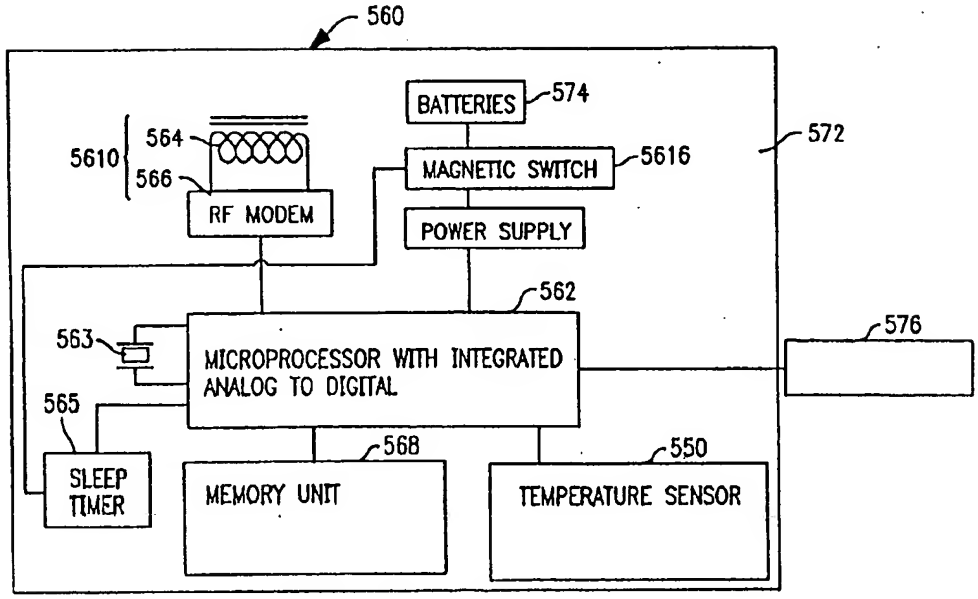

560
BATTERIES
574
572
5610
564
566
RF MODEM
MAGNETIC SWITCH
5616
POWER SUPPLY
562
576
563
MICROPROCESSOR WITH INTEGRATED ANALOG TO DIGITAL
565
SLEEP TIMER
568
MEMORY UNIT
550
TEMPERATURE SENSOR

FIG. 56

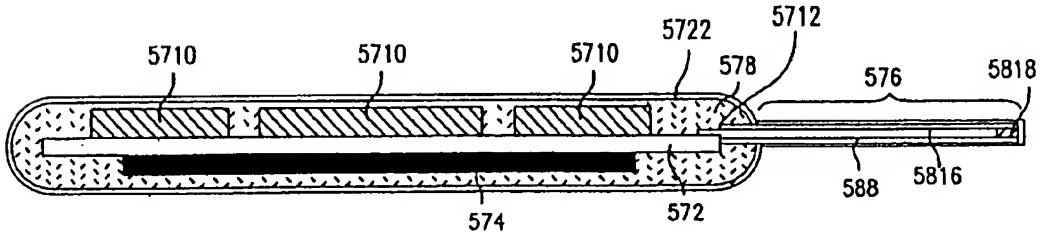

5710
5710
5710
5722
5712
578
576
5818
588
5816
574
572

FIG. 57

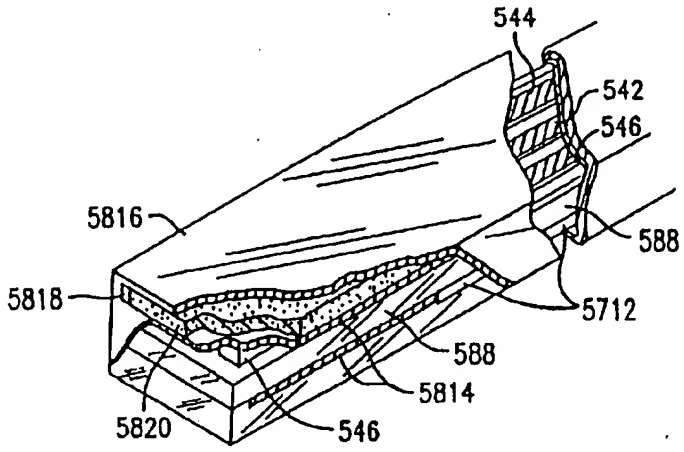

544
542
546
5816
588
5818
5712
588
5814
5820
546

FIG. 58

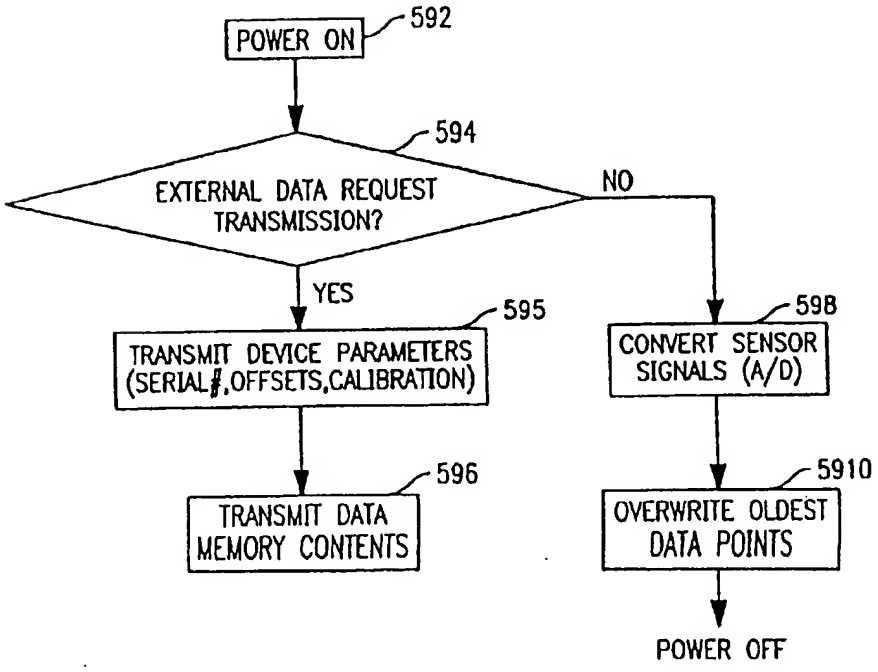

POWER ON
592
594
EXTERNAL DATA REQUEST TRANSMISSION?
NO
YES
595
TRANSMIT DEVICE PARAMETERS (SERIAL#,OFFSETS,CALIBRATION)
598
CONVERT SENSOR SIGNALS (A/D)
596
TRANSMIT DATA MEMORY CONTENTS
5910
OVERWRITE OLDEST DATA POINTS
POWER OFF

FIG. 59

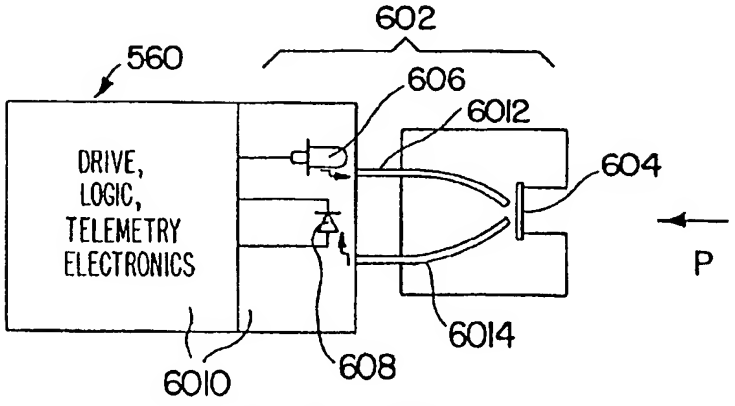


FIG. 60

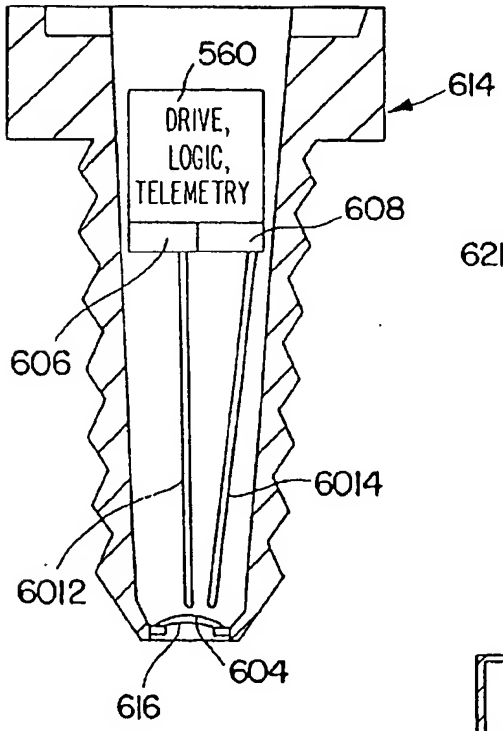


FIG. 61

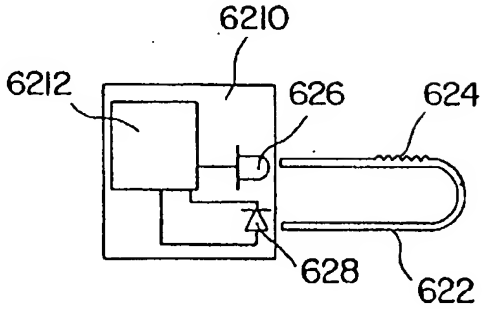


FIG. 62

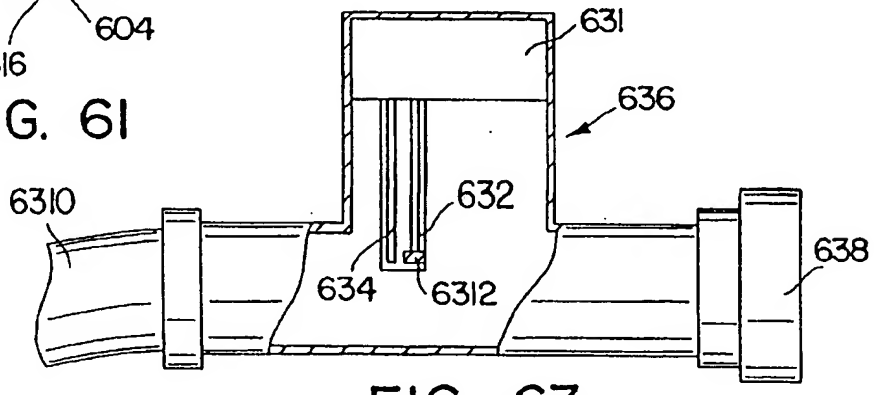


FIG. 63

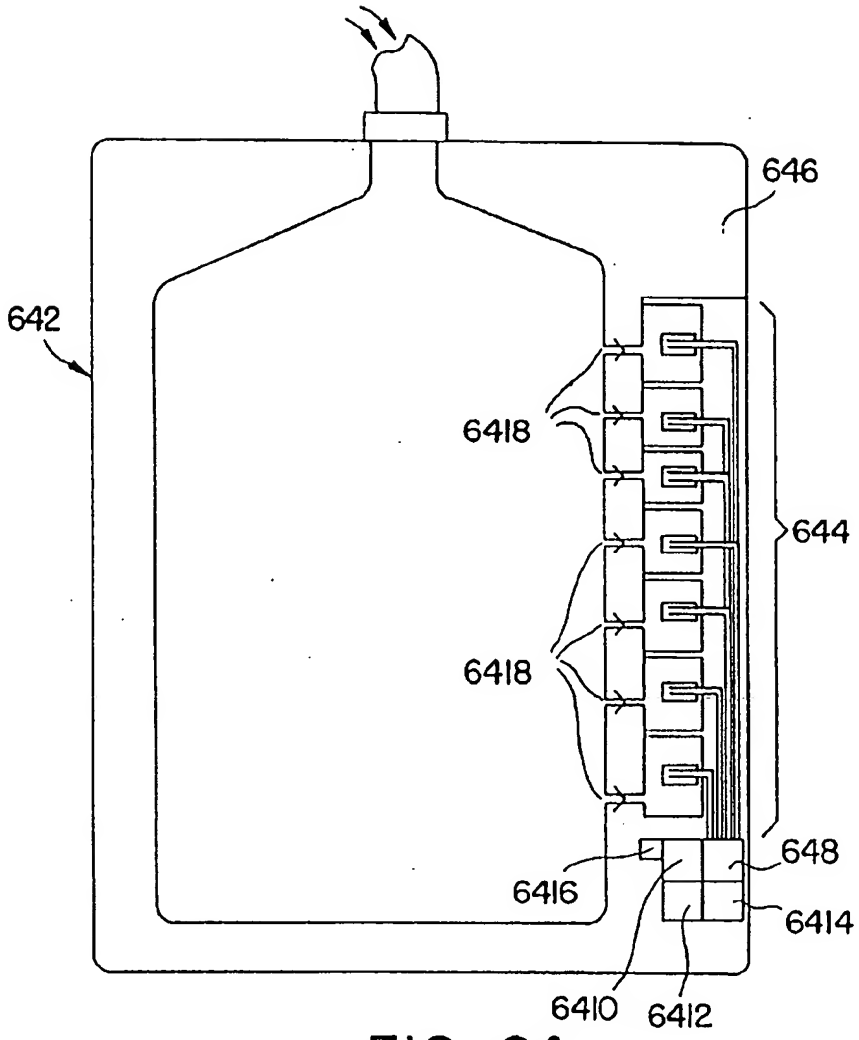


FIG. 64

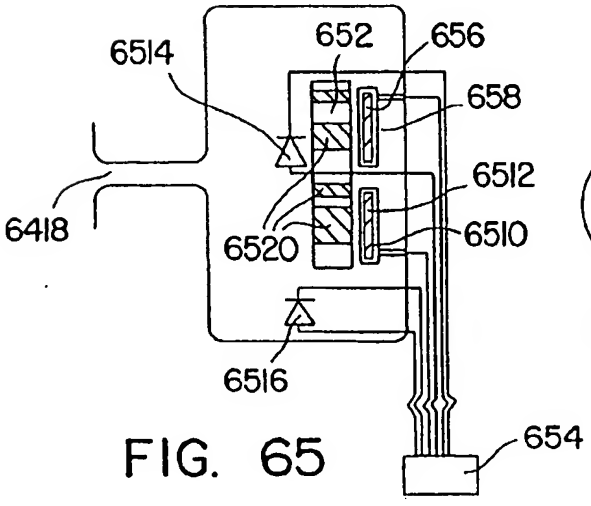


FIG. 65

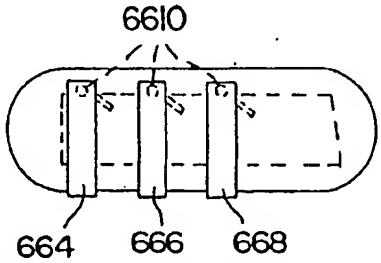


FIG. 66

MATRICES WITH MEMORIES

RELATED APPLICATIONS

This application is the National Stage of International PCT/US96/15999 which designates the U.S. and was filed on Oct. 3, 1996 and published as WO97/12680, which is a continuation-in-part of U.S. application Ser. No. 08/723,423, filed on Sep. 30, 1996, now issued as U.S. Pat. No. 5,961,923, which is a continuation-in-part of U.S. application Ser. No. 08/709,435, filed on Sep. 6, 1996, now issued as U.S. Pat. No. 6,017,496, which is a continuation-in-part of U.S. application Ser. No. 08/711,426, filed on Sep. 5, 1996, now issued as U.S. Pat. No. 6,284,459, which is a continuation-in-part of U.S. application Ser. No. 08/669,252, filed on Jun. 24, 1996, which is a continuation-in-part of U.S. application Ser. No. 08/633,410, filed on Jun. 10, 1996, now issued as U.S. Pat. No. 6,100,026, which is a continuation-in-part of International PCT application No. PCT/US96/06145 which designates the U.S. and which was filed on Apr. 25, 1996 and published as WO 96/36436, and U.S. application Ser. No. 08/639,813, filed Apr. 2, 1996, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 08/567,746, filed Dec. 5, 1995, now issued as U.S. Pat. No. 6,025,129, which is a continuation-in-part of U.S. application Ser. No. 08/538,387, filed Oct. 3, 1995, now issued as U.S. Pat. No. 5,874,214, which is a continuation-in-part of each of U.S. application Ser. Nos. 08/480,147, 08/484,486, 08/484,504, now issued as U.S. Pat. No. 5,751,629, Ser. No. 08/480,196, now issued as U.S. Pat. No. 5,925,562, and Ser. No. 08/473,660, all filed on Jun. 7, 1995, which are all continuations-in-part of U.S. application Ser. No. 08/428,662, filed Apr. 25, 1995, now issued as U.S. Pat. No. 5,741,462.

The subject matter of each of above-noted U.S. applications and International PCT applications is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to the application of information and data storage and retrieval technology to molecular tracking and identification and to biological, chemical, immunological and biochemical assays.

BACKGROUND OF THE INVENTION

Automated identification of articles using bar codes in the availability of the integrated circuit technology and computing power at reasonable costs. Such codes are typically used to track and identify consumer goods and other articles of manufacture. One of the first scanners capable of reading a bar code was installed at a supermarket in 1974, and by 1980 more than 90% of all grocery items carried a bar code by 1980. By December 1985, more than 12,000 grocery stores were equipped with scanner checkout systems [See, e.g., Harmon et al. (1989) *Reading Between the Lines-An Introduction to Bar Code Technology*, Helmers Publishing, Inc. 1989]. Bar codes have also been used in other applications, including other inventory control systems and for identification and characterization of responses to mass advertising efforts.

By electro-optically scanning the symbol on an item and generating a corresponding signal, it is possible in an associated computer whose memory has digitally stored therein the full range of items, to compare the signal derived from the scanned symbol with the stored information. When a match is found, the identity of the item and associated

information, such as, in the instance of consumer goods, its price. Thus computer technology is exploited to facilitate identification procedures using machine-readable identifiers.

Bar codes are typically read using lasers that scan from left to right, right to left, or in both directions (or other directions) across a field of alternating dark bars and reflective spaces of varying widths. Multiple scans are typically employed to minimize data errors. Because of the multiplicity of bars and spaces required for each alphanumeric character, bar codes generally require a relatively large space to convey a small amount of data. For instance, each character in the bar code system known as Code 39 requires five bars and four spaces. A high density Code 39 field corresponds to only 9.4 characters per inch. Universal Product Codes (UPCs) are another common bar code used primarily in the retail grocery trade and contain a relatively large number of bars and spaces which allow for error checking, parity checking and reduction of errors caused by manual scanning of articles in grocery stores. They accordingly require even larger space for conveyance of character information. The Codabar code, which has been developed by Pitney Bowes and is used in retail price labeling systems and by Federal Express, is a self-checking code. Each character is represented by a stand-alone group of four bars and three interleaving spaces. Federal Express uses an eleven digit Codabar symbol on each airbill to process more than 450,000 packages per night. Other codes use varying bar and space techniques to represent characters. Because of error checking requirements and for other reasons, however, the space required to place a bar code on an article is relatively large.

In addition to the large surface area required for the series of bars and spaces that form a typical bar code symbol, the code must be placed on a background that has a high reflectance level. The high level of contrast, or reflectivity ratio, between the dark bars and the reflective spaces, allows the optical sensor in the reader to discern clearly and dependably the transitions between the bars and spaces in the symbol. Ideally, the printed bar should be observed as perfectly black and the spaces should be perfectly reflective. Because those ideal conditions are seldom possible, the industry typically requires that labeling media reflect at least 70% of incident light energy. Surface reflectivity and thus quality of the media on which the bar code is placed directly affects the successful use of the bar code on that media. Additionally, the media cannot be overly transparent or translucent, since those characteristics can attenuate reflected light. Accordingly, only limited types of highly reflective media may be used for placement of bar codes. Space requirements for bar codes further include a "quiet zone" that surrounds the field of bars and spaces. In many codes, this quiet zone constitutes a border around the code symbol, thus requiring even more space for the bar code.

Bar coding also requires very precise print methods. Assuming that the printing operation is capable of printing the required density to achieve the 70% reflectance ratio, careful attention must be paid to additional major factors that influence the bar code effectiveness. These factors include ink spread/shrinkage; ink voids/specks, ink smearing; non-uniformity of ink; bar/space width tolerances; edge roughness and similar factors that must be closely controlled to ensure that the symbol will be easily scannable. In other words, the printer must pay careful attention to using paper or other media that displays the correct absorption properties properly inking the ribbon; carefully controlling hammer pressure; keeping the printhead and paper clean; properly wetting the paper and curing the ink; and maintaining proper

adjustment of the printhead control mechanism. These printing details create additional problems and expenses, particularly for placement of bar code symbols on smaller items such as coupons and mail pieces.

"Bar codes" containing an array of marks of any desired size and shape that are arranged in a reference context or frame of one or more columns and one or more rows, together with a reference marker and a reference cue have also been developed [see, U.S. Pat. No. 5,128,528]. The number of rows corresponds to the number of characters contained in the symbology selected for the array. For example, an array that is capable of conveying all the letters of the English language and ten numeral symbols could use 36 rows. The number of columns in the matrix could corresponds to the number of characters desired to be conveyed. The roles of the rows and columns in the reference frame may be reversed if desired. In the preferred embodiment, each column contains one or more dots corresponding to the character which is desired to be conveyed in that column. The reference marker and reference cue may be formed of one shape, of two marks, or according to any other desired arrangement that allows interpretation of the matrix at any desired attitude with respect to the imaging equipment. The reference cue may form a part of the reference marker, or an information dot, if desired.

Thus, there are numerous types of bar codes, codes and methodologies for use available. Bar coding and other coding technology, however, remains to be fully exploited in areas outside the consumer products domain. Furthermore, other types of optical memories have not been exploited in any industry.

Drug Discovery

Drug discovery relies on the ability to identify compounds that interact with a selected target, such as cells, an antibody, receptor, enzyme, transcription factor or the like. Traditional drug discovery relied on collections or "libraries" obtained from proprietary databases of compounds accumulated over many years, natural products, fermentation broths, and rational drug design. Recent advances in molecular biology, chemistry and automation have resulted in the development of rapid, High throughput screening (HTS) protocols to screen these collection. In connection with HTS, methods for generating molecular diversity and for detecting, identifying and quantifying biological or chemical material have been developed. These advances have been facilitated by fundamental developments in chemistry, including the development of highly sensitive analytical methods, solid state chemical synthesis, and sensitive and specific biological assay systems.

Analyses of biological interactions and chemical reactions, however, require the use of labels or tags to track and identify the results of such analyses. Typically biological reactions, such as binding, catalytic, hybridization and signaling reactions, are monitored by labels, such as radioactive, fluorescent, photoabsorptive, luminescent and other such labels, or by direct or indirect enzyme labels. Chemical reactions are also monitored by direct or indirect means, such as by linking the reactions to a second reaction in which a colored, fluorescent, chemiluminescent or other such product results. These analytical methods, however, are often time consuming, tedious and, when practiced in vivo, invasive. In addition, each reaction is typically measured individually, in a separate assay. There is, thus, a need to develop alternative and convenient methods for tracking and identifying analytes in biological interactions and the reactants and products of chemical reactions.

Combinatorial libraries

The provision and maintenance of compounds to support HTS have become critical. New and mthods for the lead generation and lead optimization have emerged to address this need for diversity. Among these methods is combinatorial chemistry, which has become a powerful tool in drug discovery and materials science. Methods and strategies for generating diverse libraries, primarily peptide- and nucleotide-based oligomer libraries, have been developed using molecular biology methods and/or simultaneous chemical synthesis methodologies [see, e.g., Dower et al. (1991) *Annu. Rep. Med. Chem.* 26:271–280; Fodor et al. (1991) *Science* 251:767–773; Jung et al. (1992) *Angew. Chem. Ind. Ed. Engl.* 31:367–383; Zuckerman et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:4505–4509; Scott et al. (1990) *Science* 249:386–390; Devlin et al. (1990) *Science* 249:404–406; Cwirla et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6378–6382; and Gallop et al. (1994) *J. Medicinal Chemistry* 37:1233–1251]. The resulting combinatorial libraries potentially contain millions of pharmaceutically relevant compounds and that can be screened to identify compounds that exhibit a selected activity.

The libraries fall into roughly three categories: fusion-protein-displayed peptide libraries in which random peptides or proteins are presented on the surface of phage particles or proteins expressed from plasmids; support-bound synthetic chemical libraries in which individual compounds or mixtures of compounds are presented on insoluble matrices, such as resin beads [see, e.g., Lam et al. (1991) *Nature* 354:82–84] and cotton supports [see, e.g., Eichler et al. (1993) *Biochemistry* 32:11035–11041]; and methods in which the compounds are used in solution [see, e.g., Houghten et al. (1991) *Nature* 354:84–86, Houghten et al. (1992) *BioTechniques* 313:412–421; and Scott et al. (1994) *Curr. Opin. Biotechnol.* 5:40–48]. There are numerous examples of synthetic peptide and oligonucleotide combinatorial libraries. The present direction in this area is to produce combinatorial libraries that contain non-peptidic small organic molecules. Such libraries are based on either a basis set of monomers that can be combined to form mixtures of diverse organic molecules or that can be combined to form a library based upon a selected pharmacophore monomer.

There are three critical aspects in any combinatorial library: (i) the chemical units of which the library is composed; (ii) generation and categorization of the library, and (iii) identification of library members that interact with the target of interest, and tracking intermediary synthesis products and the multitude of molecules in a single vessel. The generation of such libraries often relies on the use of solid phase synthesis methods, as well as solution phase methods, to produce collections containing tens of millions of compounds that can be screened in diagnostically or pharmacologically relevant in vitro assay systems. In generating large numbers of diverse molecules by stepwise synthesis, the resulting library is a complex mixture in which a particular compound is present at very low concentrations, so that it is difficult or impossible to determine its chemical structure. Various methods exist for ordered synthesis by sequential addition of particular moieties, or by identifying molecules based on spacial positioning on a chip. These methods are cumbersome and ultimately impossible to apply to highly diverse and large libraries. Identification of library members that interact with a target of interest, and tracking intermediary synthesis products and the multitude of molecules in a single vessel is also a problem. While considerable efforts have been devoted to the development of solid support chemistry, the choice of methods for structural elucidation

has been limited to spatial addressing, mixture deconvolution, direct microanalysis and chemical tagging [see, e.g., Metzger et al. (1994) *Jung, Anal. Biochem.* 219:261; Brown et al. (1995) *Mol. Diversity* 1:4; Youngquist et al. (1995) *J. Am. Chem. Soc.* 177:3900; Brummel et al. (1194) *Science* 264:399: Brenner et al. (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89: 5381; Needles et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:10700; Ohimeyer et al. *Proc. Natl. Acad. Sci. U.S.A.* 90: 10922; Eckes (1994) *Angew. Chem. Int. Ed. Engl.* 33:1573; Ni et al. (1996) *J. Med. Chem.* 39:1601]. Tagging, especially non-chemical, non-invasive tagging, is potentially the most efficient and reliable structural tracking method.

High Throughput Screening

In addition, exploitation of this diversity requires development of methods for rapidly screening compounds. Advances in instrumentation, molecular biology and protein chemistry and the adaptation of biochemical activity screens into microplate formats, has made it possible to screen of large numbers-of compounds. Also, because compound screening has been successful in areas of significance for the pharmaceutical industry, high throughput screening (HTS) protocols have assumed importance. Presently, there are hundreds of HTS systems operating throughout the world, which are used, not only for compound screening for drug discovery, but also for immunoassays, cell-based assays and receptor-binding assays.

An essential element of high throughput screening for drug discovery process and areas in which molecules are identified and tracked, is the ability to extract the information made available during synthesis and screening of a library, identification of the active components of intermediary structures, and the reactants and products of assays. While there are several techniques for identification of intermediary products and final products, nanosequencing protocols that provide exact structures are only applicable on mass to naturally occurring linear oligomers such as peptides and amino acids. Mass spectrographic [MS] analysis is sufficiently sensitive to determine the exact mass and fragmentation patterns of individual synthesis steps, but complex analytical mass spectrographic strategies are not readily automated nor conveniently performed. Also, mass spectrographic analysis provides at best simple connectivity information, but no stereoisomeric information, and generally cannot discriminate among isomeric monomers. Another problem with mass spectrographic analysis is that it requires pure compounds; structural determinations on complex mixtures is either difficult or impossible. Finally, mass spectrographic analysis is tedious and time consuming. Thus, although there are a multitude of solutions to the generation of libraries and to screening protocols, there are no ideal solutions to the problems of identification, tracking and categorization.

These problems arise in any screening or analytical process in which large numbers of molecules or biological entities are screened. In any system, once a desired molecule(s) has been isolated, it must be identified. Simple means for identification do not exist. Because of the problems inherent in any labeling procedure, it would be desirable to have alternative means for tracking and quantitating chemical and biological reactions during synthesis and/or screening processes, and for automating such tracking and quantitating.

Therefore, it is an object herein to provide methods for identification, tracking and categorization of the components of complex mixtures of diverse molecules. It is also an object herein to provide products for such identification, tracking and categorization and to provide assays, diagnostics and screening protocols that use such products. It is of particular interest herein to provide means to track and identify compounds and to perform HTS protocols.

SUMMARY OF THE INVENTION

Combinations of matrix materials with programmable data storage or recording devices or other memory means, herein referred to as memories, and assays using these combinations are provided. These combinations are referred to herein as matrices with memories. Protocols in which all steps, including synthesis and screening or assaying, are performed on a single platform are provided herein. In addition protocols in which a series of matrices with memories are used and information is transferred from one memory to another are also provided.

The memories with matrices may also include a sensing function that records information related to the sample and/or source of the sample and/or to detect or sense and store information about events in proximity the memory. In particular, sensors with memories are provided.

By virtue of the memory with matrix combination, molecules, such as antigens, antibodies, ligands, proteins and nucleic acids, and biological particles, such as phage and viral particles and cells, that are associated with, such as in proximity to or in physical contact with the matrix combination or linked via information stored in a remote computer, can be electromagnetically tagged by programming the memory with data corresponding to identifying information or can be tagged by imprinting or encoding the matrix with identifying information. In particular, optical memory means and the use thereof are provided herein.

Also provided herein are sensors that combine memories with matrices and sensors. Programming and reading the memory is effected remotely, preferably electromagnetic radiation, particularly radio frequency [RF] or radar, microwave, or microwave or energies between RF and microwave, or by reading the imprinted information.

Optical memories, either bar coded information, particularly the 2-D bar codes provided herein, or optically encoded memories, such as memories that rely on changes in chemical or physical properties of particular molecules are contemplated herein. Memories may also be remote from the matrix, such as instances in which the memory device is precoded with a mark or identifier or the matrix is encoded with a bar code. The identity [i.e., the mark or code] of each device is written to a memory, which may be a computer or a piece of paper or any recording device, and information associated with each matrix is stored in the remote memory and linked to or associated with the code or other identifier.

Of particular interest herein are matrices with memories in which the matrices have an engraved code. These matrices with memories are herein referred to as matrices with codes or optical memory devices [OMDs]. The memories are remote recording devices, such as a remote computer memory in which information associated with the codes is stored. The materials are encoded with identifying information and/or any other information of interest. Synthetic protocols and assays using encoded matrix materials are provided. By virtue of this code on the matrix, molecules, such as antigens, antibodies, ligands, proteins and nucleic acids, and biological particles, such as phage and viral particles and cells, that are associated with, such as in proximity to or in physical contact with the matrix, can be tagged by programming a memory, such as a memory in a computer, with data corresponding to the encoded identify-

ing information. The identity [i.e., the mark or code] of each device is written to a memory, which may be a computer or a piece of paper or any recording device, and information associated with each matrix is stored in the remote memory and linked to the code or other identifier.

The molecules and biological particles that are associated with the matrix combination, such as in proximity to or in physical contact or with the matrix combination, can be identified and the results of the assays determined by retrieving the stored data points from the memories. Querying the memory will identify associated molecules or biological particles that have reacted.

In certain embodiments of the matrices with memories, reactions, assays and other events or external parameters, such as temperature and/or pH, can be monitored because occurrence of a reaction or an event can be detected and such detection sent to the recording device when proximate to the matrix and recorded in the memory.

The combinations provided herein thus have a multiplicity of applications, including combinatorial chemistry, isolation and purification of target macromolecules, capture and detection of macromolecules for analytical purposes, high throughput screening, selective removal of contaminants, enzymatic catalysis, drug delivery, chemical modification, information collection and management and other uses. These combinations are particularly advantageous for use in multianalyte analyses, assays in which a electromagnetic signal is generated by the reactants or products in the assay, for use in homogeneous assays, and for use in multiplexed protocols.

In preferred embodiments, these matrix with memory combinations contain (i) a miniature recording device that includes one or more programmable data storage devices [memories] or an engraved or imprinted optically readable code or a 3-D optical memory, that can be remotely read and in preferred embodiments also remotely programmed; and (ii) a matrix, such as a particulate support used in chemical syntheses. These matrix with memory combinations or the memories also are combined with sensors.

The matrix materials [matrices] are any materials that are routinely used in chemical and biochemical synthesis. The matrix materials are typically polymeric materials that are compatible with chemical and biological syntheses and assays, and include, glasses, silicates, celluloses, polystyrenes, polysaccharides, polypropylenes, sand, and synthetic resins and polymers, including acrylamides, particularly cross-linked polymers, cotton, and other such materials. The matrices may be in the form of particles or may be continuous in design, such as a test tube or microplate, 96 well or 384 well or higher density formats or other such microplates and microtiter plates. The matrices may contain one or a plurality of recording devices. For example, each well or selected wells in the microplate include a memory device in contact therewith or embedded therein. The plates may further contain embedded scintillant or a coating of scintillant [such as FlashPlate™, available from DuPont NEN®, and plates available from Packard, Meriden, Conn. and Cytostar-T plates available from Amersham International plc, U.K.]. Automated robotic protocols will incorporate such plates for automated multiplexing [performing a series of coupled synthetic and processing steps, typically, though not necessarily on the same platform, i.e. coupling of the chemistry to the biology] including one or more of the following, synthesis, preferably accompanied by writing to the linked memories to identify linked compounds, screening, including using protocols with matrices with memories, and compound identification by querying the memories of matrices associated with the selected compounds.

The matrices are either particulate of a size that is roughly about 1 to 20 mm^3 [or 1–20 mm in its largest dimension], preferably about 10 mm^3 or smaller, preferably 1 mm^3 or smaller, such as minute particulates, or a continuous medium, such as a microtiter plate, or other multi-well plate, or plastic or other solid polymeric vial or glass vial or catheter-tube [for drug delivery] or such container or device conventionally used in chemistry and biological syntheses and reactions.

In instances in which the matrix is continuous, the data storage device [memory] may be placed in, on, or under the matrix medium or may be embedded in the material of the matrix or removably attached, such as in a sleeve designed to fit on the matrix.

Plates that include a bar code, particularly the two-dimensional optical bar code provided herein on the base of each well or elsewhere. The two-dimensional bar code or other such code is particularly suited for application to each well in a microplate, such as a microtiter plate, that contain 96, 384, 1536 or higher density formats. The bar code may also be used in combination with modules that are fitted into the frames of 96 wells, or higher density formats [such as those available from NUNC, such as NUNC-Immuno Modules, and also sources, such as COSTAR plate strips, Cytostar-T plates from Amersham International plc, U.K., and Octavac Filter Strips]. Separate containers or strips of containers are designed to fit into microplate frames. Each such container may be encoded with a bar code so that, upon removal from the strip, the container, and thereby, its contents or history, may be identified.

In embodiments herein in which the matrices with memories are used in assays, such as scintillation proximity assays [SPA], FP [fluorescence polarization] assays, FET [fluorescent energy transfer] assays, FRET [fluorescent resonance energy transfer] assays and HTRF [homogeneous time-resolved fluorescence] assays, the matrices may be coated with, embedded with or otherwise combined with or in contact with assay material, such as scintillant, fluophore or other fluorescent label. The resulting combinations are called luminescing memories with matrices. When used in SPA formats they are referred to as scintillating matrices with memories and when used in non-radioactive energy transfer formats [such as HTRF] they are referred to as fluorescing memories with matrices.

The recording device used in proximity to the matrix is preferably a miniature device or is part of the support, such as an optical memory, typically less than 10–20 mm^3 [or 10–20 mm in its largest dimension] in size, preferably smaller, such as 1 to 5 mm, that includes at least one data storage unit that includes a remotely programmable and remotely readable, preferably non-volatile, memory. This device with remotely programmable memory is in proximity to, associated with or in contact with the matrix. In particular, the recording device includes a memory device, preferably having memory means, preferably non-volatile, for storing a plurality of data points and means for receiving a transmitted signal that is received by the device and for causing a data point corresponding to the data signal to be permanently stored within the memory means. If needed, the recording device further includes a shell [coating] that is non-reactive with and impervious to any processing steps or solutions in which the combination of matrix with recording device [matrix with memory] is placed, and that is trans-

missive of read or write signals transmitted to the memory. The device may also include at least one support matrix disposed on an outer surface of the shell for retaining molecules or biological particles. The shell and support matrix may be the same. In such instances, the shell must be treated or derivatized such that molecules, particularly amino acids and nucleic acids, can be linked, preferably either electrostatically or covalently, thereto. Thus, a transponder enclosed in plastic, must be further treated or coated to render it suitable for linkage of the molecule or biological particle. In preferred embodiments provided herein, the device also includes a photodetector to detect events and means to write a record of the event to the memory.

The data storage device or memory is programmed with or encoded with information that identifies molecules or biological particles, either by their process of preparation, their identity, their batch number, category, physical or chemical properties, combinations of any of such information, or other such identifying information. The molecules or biological particles are in physical contact, direct or indirect, or in proximity with the matrix, which in turn is in physical contact or in the proximity of the recording device that contains the data storage memory. The molecule or biological particle may also be associated, such that a molecule or biological particle that had been linked to or in proximity with a matrix with memory may be identified [i.e., although the matrix particle and biological particle or molecule are not linked or in proximity, the identify of the matrix that had been linked to the molecule or particle is known]. Typically, the matrix is on the surface of the recording device and the molecules and biological particles are in physical contact with the matrix material. In certain embodiments, the memory device may be linked to or in proximity to more than one matrix particle.

The data storage device or memory can also be programmed by virtue of a reaction in proximity to or in the vicinity of the matrix with memory. In particular, the recording devices include memories and also additional components that detect occurrence of external events or to monitor the status of external parameters, such as EM emissions, changes in temperature or pH, ion concentrations and other such solution parameters. For example, recording devices include memories and also include a photodetector can detect the occurrence of fluorescence or other optical emission. Coupling this emission with an amplifier and providing a voltage to permit data storage in the matrix with memory during the reaction by way of, for example an RF signal transmitted to and received by an antenna/rectifier combination within the data storage device or providing voltage sufficient to write to memory from a battery [see, e.g., U.S. Pat. No. 5,350,645 and U.S. Pat. No. 5,089,877], permits occurrence of the emission to be recorded in the memory.

The recording device [containing the memory] is associated with the memory. Typically, the recording device is coated with at least one layer of material, such as a protective polymer or a glass, including polystyrene, heavy metal-free glass, plastic, ceramic, and may be coated with more than one layers of this and other materials. It must be treated to render it suitable for linking molecules or biological particles when it is used as a support. For example, it may be coated with a ceramic or glass that are suitably deivatized or then coated with or linked to the matrix material. Alternatively, the glass or ceramic or other coating may serve as the matrix. In other embodiments the recording device and the matrix material are in proximity, such as in a container of a size approximately that of the device and matrix material. In yet other embodiments the recording device and matrix material are associated, such that the molecule or biological particle that was linked to the matrix or that was in proximity thereto may be identified.

The matrix combinations [the matrices with memories], thus, contain a matrix material, typically in particulate form, in physical contact with a tiny device containing one or more remotely programmable data storage units [memories]. Contact can be effected by placing the recording device with memory on or in the matrix material or in a solution that is in contact with the matrix material or by linking the device, either by direct or indirect covalent or non-covalent interactions, chemical linkages or by other interactions, to the matrix. Alternatively, matrices with memories carry a code, such as a bar code, preferably a two-dimensional bar code, on typically one surface and the memory is remote, such as a memory in a computer or any written record by which the code can be deciphered and information stored and associated therewith.

For example, when the memories are proximate to the matrix, contact can be effected chemically, by chemically coupling the recording device with memory to the matrix, or physically by coating the recording device with the matrix material or another material, by physically inserting or encasing the device in the matrix material, by placing the device onto the matrix or by any other means by which the device can be placed in contact with or in proximity to the matrix material. The contact may be direct or indirect via linkers. The contact may be effected by absorption or adsorption.

Since matrix materials have many known uses in conjunction with molecules and biological particles, there are a multitude of methods known to artisans of skill in this art for linking, joining or physically contacting the molecule or biological particle with the matrix material. In some embodiments, the recording device with data storage unit is placed in a solution or suspension of the molecule or biological particle of interest. In some of such instances, the container, such as the microtiter plate or test tube or other vial, is the matrix material. The recording device is placed in or on the matrix or is embedded, encased or dipped in the matrix material or otherwise place in proximity by enclosing the device and matrix material in a sealed pouch or bag or container [MICROKAN™] fabricated from, preferably, porous material, such as polytetrafluoroethylene [marketed under the trademark TEFLON® (Trademark, E. I. DuPont)] or polypropylene prepared with pores, that is inert to the reaction of interest and that have pores of size permeable to desired components of the reaction medium.

More than one data storage device or engraved coded or combination thereof may be in proximity to or contact with a matrix particle, or more than one matrix particle may be in contact with on device. For example, microplates, such as microtiter y plates or other such high density format [i.e. 96, 384 1536 or more wells per plate, such as those available from Nunc, Naperville, Ill., Costar, Cambridge Mass. and Millipore, Bedford, Mass.] with the recording device containing the data storage unit [remotely programmable memory] embedded in each well or vials [typically with a 1.5 ml or smaller capacity] with an embedded recording device may be manufactured.

In a preferred embodiment, the recording device is a semiconductor that is approximately 10 mm or less in its largest dimension and the matrix material is a particle, such as a polystyrene bead. The device and a plurality of particles, referred to as "beads", typically about 1 mg to about 50 mg, but larger size vessels and amounts up to 1000 mg, prefer-

ably 50 to about 200 mg, are sealed in chemically inert porous supports, such as polypropylene formed so that it has pores of a selected size that excludes the particles but permits passage of the external medium. For example, a single device and a plurality of particles may be sealed in a porous or semi-permeable inert material to produce a microvessel [such as the MICROKAN™] such as a TEFLON® [polytetrafluoroethylene] or polypropylene or membrane that is permeable to the components of the medium, or they may be contained in a small closable container that has at least one dimension that is porous or is a semi-permeable tube. Typically such tube, which preferably has an end that can be opened and sealed or closed tightly.

These microvessels preferably have a volume of about 200–500 mm^3, but can have larger volumes, such as greater than 500 mm^3 [or 1000 mm^3] at least sufficient to contain at least 200 mg of matrix particles, such as about 500–3000 mm^3, such as 1000–2000 or 1000 to 1500, with preferred dimensions of about 1–10 mm in diameter and 5 to 20 mm in height, more preferably about 5 mm by 15 mm, or larger, such as about 1–6 cm by 1–6 cm. The porous wall should be non-collapsible with a pore size in the range of 70 μM to about 100 μM, but can be selected to be semi-permeable for selected components of the medium in which the microvessel is placed. The preferred geometry of these combinations is cylindrical. These porous microvessels may be sealed by heat or may be designed to snap or otherwise close. In some embodiments they are designed to be reused. In other embodiments, the microvessel MICROKAN™ with closures may be made out of non-porous material, such as a tube in the conical shape or other geometry.

Such vessels thus are relatively rigid containers with mesh side walls. Typically, a single compound is synthesized in each one, and each one contains a unique memory with encoded information or a read/write memory and are designed to be loaded with solid phase resin. Syntheses takes place by allowing reagents to flow through the outer mesh walls. The preferred embodiment has a volume of about 330 μl of which approximately 200 μl is available for resin with the remainder of the space being occupied by the RF tag. In other embodiments the microvessel is engraved with the 2-D optical code provided herien. Typically about 30 mg of most commercial resins can be loaded into this volume leaving enough space available for the resin to swell and still remain loose within the walls.

Also provided herein are tubular devices (or other geometry) in which the recording device is enclosed in a solid polymer, such as a polypropylene, which is then radiation grafted with selected monomers to produce a surface suitable for chemical synthesis and linkage of molecules or biological particles. These tubular devices (or other geometry), such as the MICROTUBE™ microvessels, may contain a recording device or may include a code engraved, such as by a laser, or otherwise imprinted on the surface. The tubular devices are preferably TEFLON® (polytetrafluoroethylene (PTFE)), polyethylene, high density polyethylene, polypropylene, polystyrene, polyester, ceramic, composites of any of these materials and other such materials. A method for radiation grafting of monomers to PTFE is provided herein. These devices may also be formed from a ball with a screw cap (MICROBALLS™) or other type of cap to permit access to the inside.

These types of memories with matrices are polypropylene or fluoropolymer tubes with a radiation grafted functionalized polystyrene surface that completely enclose a selected memory, such as an RF tag. Syntheses are performed on the functionalized polystyrene surface. These devices can be used for solid phase chemistry without the need to load solid phase resins.

Other devices of interest, are polypropylene supports, generally about 5–10 mm in the largest dimension, and preferably a cube or other such shape, that are marked with a code, and tracked using a remote memory. These microvessels can be marked with a code, such as a bar code, alphanumeric code, the 2-D optical bar code provided herein, or other mark or include an optical memory, for identification, particularly in embodiments in which the memory is not in proximity to the matrix, but is remote therefrom and used to store information regarding each coded vessel.

The combination of matrix with memory is used by contacting it with, linking it to, or placing it in proximity with a molecule or biological particle, such as a virus or phage particle, a bacterium or a cell, to produce a second combination of a matrix with memory and a molecule or biological particle. In certain instances, such combinations of matrix with memory or combination of matrix with memory and molecule or biological particle may be prepared when used or may be prepared before use and packaged or stored as such for futures use. The matrix with memory when linked or proximate to a molecule or biological particle is herein referred to as a microreactor.

The miniature recording device containing the data storage unit(s) with remotely programmable memory, includes, in addition to the remotely programmable memory, means for receiving information for storage in the memory and for retrieving information stored in the memory. Such means is typically an antenna, which also serves to provide power in a passive device when combined with a rectifier circuit to convert received energy, such as RF, into voltage, that can be tuned to a desired electromagnetic frequency to program the memory. Power for operation of the recording device may also be provided by a battery attached directly to the recording device, to create an active device, or by other power sources, including light and chemical reactions, including biological reactions, that generate energy.

Preferred frequencies are any that do not substantially alter the molecular and biological interactions of interest, such as those that are not substantially absorbed by the molecules or biological particles linked to the matrix or in proximity of the matrix, and that do not alter the support properties of the matrix. Radio frequencies are presently preferred, but other frequencies, such as radar, or optical lasers will be used, as long as the selected frequency or optical laser does not interfere with the interactions of the molecules or biological particles of interest. Thus, information in the form of data points corresponding to such information is stored in and retrieved from the data storage device by application of a selected electromagnetic radiation frequency, which preferably is selected to avoid interference from any background electromagnetic radiation.

The preferred miniature recording device for use in the combinations herein is a single substrate of a size preferably less than about 10 to 20 mm^3 [or 10–20 mm in its largest dimension, most preferably 2 mm or less], that includes a remotely programmable data storage unit(s) [memory], preferably a non-volatile memory, and an antenna for receiving or transmitting an electromagnetic signal [and in some embodiments for supplying power in passive devices when combined with a rectifier circuit] preferably a radio frequency signal; the antenna, rectifier circuit, memory and other components are preferably integrated onto a single

substrate, thereby minimizing the size of the device. An active device, i.e., one that does not rely on external sources for providing voltage for operation of the memory, may include a battery for power, with the battery attached to the substrate, preferably on the surface of the substrate. Vias through the substrate can then provide conduction paths from the battery to the circuitry on the substrate. The device is rapidly or substantially instantaneously programmable, preferably in less than 5 seconds, more preferably in about 1 second, and more preferably in about 50 to 100 milliseconds or less, and most preferably in about 1 millisecond or less. In a passive device that relies upon external transmissions to generate sufficient voltage to operate, write to and read from an electronic recording device, the preferred memory is non-volatile, and may be permanent. Such memories may rely antifuse-based architecture or flash memory. Other memories, such as electrically programmable erasable read only memories [EEPROMs] based upon other architectures also can be used in passive devices. In active recording devices that have batteries to assure continuous power availability, a broader range of memory devices may be used in addition to those identified above. These memory devices include dynamic random access memories [DRAMS, which refer to semiconductor volatile memory devices that allow random input/output of stored information; see, e.g., U.S. Pat. Nos. 5,453,633, 5,451,896, 5,442,584, 5,442,212 and 5,440,511], that permit higher density memories, and EEPROMs. Monolithic devices, such as that described herein are among the preferred electromagnetically programmable memories.

Containers, such as vials, tubes, microtiter plates, reagent bottles, sample and collection vials, autosampler carousals, HPLC columns and other chromatography columns, such as GC columns, electrophoresis and capillary electrophoresis equipment, plate readers, reagent carriers, blood bags, fraction collectors, capsules and the like, which are in contact with a recording device that includes a data storage unit with programmable memory or include an optical memory, such as a 3-D optical memory incorporated into the material or attached to the container or instrument, or other analytical tool are also provided. The memories may also be used in combination with instruments, including, but not limited to HPLC, gas chromatographs (GC), mass spectrometers (MS), NMR instruments, GC-MS, stir bars spectrometers, including fluorimeters, luminometers, and capillary electrophoresis and electrophoresis instruments and tubes and plates used therefor. Thus, an entire laboratory may be augmented with memories linked to or proximate to every container, instrument, and device, from reagent bottle to collected fraction, used in a particular protocol, whereby a sample may be tracked. The information that is stored will include information regarding the identity of a sample and/or source of the sample.

A container is typically of a size used in immunoassays or hybridization reactions, generally a liter or less, typically less than 100 ml, and often less than about 10 ml in volume, typically 100 μl–500 μl, particularly 200–250 μl. Alternatively the container can be in the form of a plurality of wells, such as a microtiter plate, each well having about 1 to 1.5 ml or less in volume. The container is transmissive to the electromagnetic radiation, such as radio frequencies, infrared wavelengths, radar, ultraviolet wavelengths, microwave frequencies, visible wavelengths, X-rays or laser light, used to program the recording device.

The memories have also been combined with stirring bars, particularly magnetic stir bars, thereby permitting remote identification of any beakers, bottles, and other containers in which stir bars are used.

Methods for electromagnetically tagging molecules or biological particles are provided. Such tagging is effected by placing the molecules or biological particles of interest in proximity with the recording device or with the matrix with memory, and programming or encoding the identity of the molecule or synthetic history of the molecules or batch number or other identifying information into the memory. The, thus identified molecule or biological particle is then used in the reaction or assay of interest and tracked by virtue of its linkage to the matrix with memory, its proximity to the matrix with memory or its having been linked or in proximity to the matrix [i.e., its association with], which can be queried at will to identify the molecule or biological particle. The tagging and/or reaction or assay protocols may be automated. Automation may use robotics [see, U.S. Pat. No. 5,463,564, which provides an automated iterative method of drug design]. In addition, methods for addressing such memories in individually among a group are provided.

In particular, methods for tagging constituent members of combinatorial libraries and other libraries or mixtures of diverse molecules and biological particles are provided. These methods involve electromagnetically tagging or optically imprinting molecules, particularly constituent members of a library, by contacting the molecules or biological particles or bringing such molecules or particles into proximity with a matrix with memory and programming the memory [by writing to it or by imprinting the matrix with an optical bar code or by associating a pre-engraved code with identifying information with retrievable information from which the identity, synthesis history, batch number or other identifying information can be retrieved. The contact is preferably effected by coating, completely or in part, the recording device with memory with the matrix and then linking, directly or via linkers, the molecule or biological particle of interest to the matrix support. The memories can be coated with a protective coating, such as a glass or silicon, which can be readily derivatized for chemical linkage or coupling to the matrix material. In other embodiments, the memories can be coated with matrix, such as for example dipping the memory into the polymer prior to polymerization, and allowing the polymer to polymerize on the surface of the memory.

In other embodiments, the memory is part of the container that contains the sample or is part of the instrument. As a sample is moved, for example, from container to container or from instrument to container to a plate, the information from one memory is transferred by reading one memory and writing to the next so the identity of the contents is tracked as it is processed. Such movement and tracking can be automated.

If the matrices are used for the synthesis of the constituent molecules, the memory of each particle is addressed and the identity of the added component is encoded in the memory at [before, during, or preferably after] each step in the synthesis. At the end of the synthesis, the memory contains a retrievable record of all of the constituents of the resulting molecule, which can then be used, either linked to the support, or following cleavage from the support in an assay or for screening or other such application. If the molecule is cleaved from the support with memory, the memory must remain in proximity to the molecule or must in some manner be traceable [i.e., associated with] to the molecule. Such synthetic steps may be automated.

In preferred embodiments, the matrix with memory with linked molecules [or biological particles] are mixed and reacted with a sample according to a screening or assay protocol, and those that react are isolated. The identity of

reacted molecules can then be ascertained by remotely retrieving the information stored in the memory and decoding it to identify the linked molecules. Such steps can be performed on a single platform or on a series of platforms in which with each transfer information from one memory is transferred to a subsequent memory that is in contact with the sample.

Compositions containing combinations of matrices with memories and compositions of matrices with memories and molecules or biological particles are also provided. In particular, optically coded or electronically tagged libraries of oligonucleotides, peptides, proteins, non-peptide organic molecules, phage display, viruses and cells are provided. Particulate matrices, such as polystyrene beads, with attached memories, and continuous matrices, such as microtiter plates or slabs or polymer, with a plurality of embedded or attached memories are provided.

These combinations of matrix materials with memories and combinations of matrices with memories and molecules or biological particles may be used in any application in which support-bound molecules or biological particles are used. Such applications include, but are not limited to diagnostics, such as immunoassays, drug screening assays, combinatorial chemistry protocols and other such uses. These matrices with memories can be used to tag cells for uses in cell sorting, to identify molecules in combinatorial syntheses, to label monoclonal antibodies, to tag constituent members of phage displays, affinity separation procedures, to label DNA and RNA, in nucleic acid amplification reactions [see, e.g., U.S. Pat. No. 5,403,484; U.S. Pat. No. 5,386,024; U.S. Pat. No. 4,683,202 and, for example international PCT Application WO/94 02634, which describes the use of solid supports in connection with nucleic acid amplification methods], to label known compounds, particularly mixtures of known compounds in multianalyte analyses], to thereby identify unknown compounds, or to label or track unknowns and thereby identify the unknown by virtue of reaction with a known. Thus, the matrices with memories are particularly suited for high throughput screening applications and for multianalyte analyses.

Systems and methods for recording and reading or retrieving the information in the data storage devices regarding the identity or synthesis of the molecules or biological particles are also provided. The systems for recording and reading data include: a host computer or other encoder/decoder instrument having a memory for storing data relating to the identity or synthesis of the molecules, and a transmitter means for receiving a data signal and generating a signal for transmitting a data signal; and a recording device that includes a remotely programmable, preferably non-volatile, memory and transmitter means for receiving a data signal and generating at least a transmitted signal and for providing a write signal to the memory in the recording device. The host computer stores transmitted signals from the memories with matrices, and decodes the transmitted information.

In particular, the systems include means for writing to and reading from the memory device to store and identify each of the indicators that identify or track the molecules and biological particles. The systems additionally include the matrix material in physical contact with or proximate to the recording device, and may also include a device for separating matrix particles with memory so that each particle or memory can be separately programmed.

Methods for tagging molecules and biological particles by contacting, either directly or indirectly, a molecule or biological particle with a recording device; transmitting from a host computer or decoder/encoder instrument to the device electromagnetic radiation representative of a data signal corresponding to an indicator that either specifies one of a series of synthetic steps or the identity or other information for identification of the molecule or biological particle, whereby the data point representing the indicator is written into the memory, are provided. Where optical memories are used the memories are optically scanned and the encoded information read.

Methods for reading identifying information from recording devices linked to or in contact with or in proximity to or that had been in contact with or proximity to a electromagnetically tagged molecule or electromagnetically tagged biological particles are provided. These methods include the step of exposing the recording device containing the memory in which the data are stored to electromagnetic radiation [EM]; and transmitting to a host computer or decoder/encoder instrument an indicator representative of a the identity of a molecule or biological particle or identification of the molecule or biological particle linked to, in proximity to or associated with the recording device.

One, two, three and N-dimensional arrays of the matrices with memories are also provided. Each memory includes a record [or for pre-encoded memories with matrices, the record is associated with code in a remote memory] of its position in the array. Such arrays may be used for blotting, if each matrix particle is coated on one at least one side with a suitable material, such as nitrocellulose. For blotting, each memory is coated on at least one side with the matrix material and arranged contiguously to adjacent memories to form a substantially continuous sheet. After blotting, the matrix particles may be separated and reacted with the analyte of interest [i., a labeled antibody or oligonucleotide or other ligand], after which the physical position of the matrices to which analyte binds may be determined. The amount of bound analyte, as well as the kinetics of the binding reaction, may also be quantified. Southern, Northern, Western, dot blot and other such assays using such arrays are provided. Dimensions beyond three can refer to additional unique identifying parameters, such as batch number, and simultaneous analysis of multiple blots.

Assays that use combinations of (i) a memory, such as a 2-D optical bar code or a miniature recording device that contains one or more programmable data storage devices [memories] that can be remotely programmed and read; and (ii) a matrix, such as a particulate support used in chemical syntheses, are provided. The remote programming and reading is preferably effected using electromagnetic radiation.

Also provided are scintillation proximity assays, HTRF, FP, FET and FRET assays in which the memories are in proximity with or are in physical contact with the matrix that contains scintillant for detecting proximate radionucleotide signals or fluorescence. In addition, embodiments that include a memory device that also detects occurrence of a reaction are provided.

Molecular libraries, DNA libraries, peptide libraries, biological particle libraries, such as phage display libraries, in which the constituent molecules or biological particles are combined with a solid support matrix that is combined with a data storage unit with a programmable memory are provided.

Affinity purification protocols in which the affinity resin is combined with a recording device containing a data storage unit with a programmable memory are also provided.

Immunological, biochemical, cell biological, molecular biological, microbiological, and chemical assays in which

memory with matrix combinations are used are provided. For example immunoassays, such as enzyme linked immunosorbent assays [ELISAs] in which at least one analyte is linked to a solid support matrix that is combined with a recording device containing a data storage unit with a programmable, preferably remotely programmable and non-volatile, memory are provided.

Of particular interest herein, are multiprotocol applications [such as multiplexed assays or coupled synthetic and assay protocols] in which the matrices with memories are used in a series [more than one] of reactions, a series [more than one] of assays, and/or a series of more or more reactions and one or more assays, typically on a single platform or coupled via automated analysis instrumentation. As a result synthesis is coupled to screening, including compound identification and analysis, where needed.

As noted above, where sample is transferred, for example, from vial or tubes to plates, etc., the vials, plates, reagent bottles and columns and other items used in drug discovery or for collecting and analyzing samples, screening and analysis equipment and instrumentation include memory, such as an RF tag, optical memory, such as a 3-D optical memory, or 2-D optical bar code. As a sample is synthesized or obtained and processed, the information is transferred from one memory to the next, thereby providing a means to track the sample and identity from synthesis to screening to analysis.

Methods for engraving bar codes, bar codes and bar-code engraved devices are also provided herein. In particular OMDs are provided and methods for writing to the surface of these devices and reading the engraved symbology are provided. The OMDs are fabricated from a suitable material, such as black, white or colored glass, TEFLON® [polytetrafluoroethylene], polyethylene, high density polyethylene, polypropylene, polystyrene, polyester, ceramic, composites of any of these materials and other such materials. The typical OMD is 10 mm or smaller in its largest dimension and is encoded by direct deposit, dot matrix deposit, direct laser write or dot matrix scan laser write. They may be precoded or coded prior to or even during use. For use in the applications provided herein, at least one surface or a portion of a surface is treated to render it suitable for use as a support, such as by grafting, ion implant, vacuum deposit, oxidation, combinations thereof, suitable derivatization or any other means known to those of skill in the art by which materials are treated to render them suitable for use as supports. The OMDs also have applications as a data pad for recording information about linked molecules or biological particles, or for monitoring storage and location, or in clinical labs for recording relevant information. The OMDs may be in the form of microplates in which each well is encoded or in combination with any instrumentation used in biological and chemical processing and screening.

Also of particular interest herein, are combinations of vials, tubes or other containers with sleeves [see, e.g., FIGS. 35–38], in which vial [such as a Hewlett Packard or Waters HPLC vial] is inserted into a fitted sleeve that contains the memory. These may be those used for patient samples or HPLC samples or other samples, such as samples from fraction collectors.

In one embodiment a carousel equipped with a reader and linked to a computer with software is also provided. Also provided herein is the carousel that houses a plurality of such vials or vessels, which are each equipped with a memory device. The carousel is mounted on a rotating seat that is designed to be rotated either manually, or by electrical, mechanical or other suitable control. This seat is mounted to a housing and is positioned such that the carousel rotates with the memory device [i.e., a read/write device] coming in proximity to a read/write controller. This read/write controller is located within the housing and positioned such that a detector head for the read/write controller is adjacent the read/write device as held in the carousel. In order to assist the accurate positioning of the carousel, a plunger is oriented on the surface of the housing to strike the carousel at the location of the vial which helps to prevent further rotation of the carousel while the read/write device is communicating with the read/write controller. The read/write controller is a micro-controller based instrument that generates a selected frequency, such as 125 khz radio frequency (RF) signal, when RF devices are used, which is transmitted to the read/write controller head which includes an antenna element that is designed to transmit the particular RF signal. It will be appreciated that other electromagnetic frequencies, such as microwave, radar, x-ray, UV, and IR may be used.

Also included in the read/write controller is an oscillator and EEPROM memory which, in combination, control a transmitter and receiver for the rf signals. The read/write device includes a semiconductor that is attached to a similarly shaped antenna designed to receive the signals transmitted from the read/write controller head. The signal from the read/write device antenna is filtered and a portion of the signal is rectified to create the power required to drive the semiconductor. Once the power is created, the semiconductor transmits through the same antenna information, such as identifying information, that has been programmed previously. This allows each vial to be attached to a read/write device, and programmed with a particular identification code or other information.

In alternative embodiments, the read/write device is preprogrammed or the container, such as the vial, reagent bottle, etc. is engraved with the 2-D optical bar code provided herein and using the methods herein. The vial can be attached to the read/write device either before or after the vial is filled. Once the vial is attached to a read/write device, the vial can be inserted into the carousel with a number of other vials similarly attached to the read/write devices.

The information that is transmitted from the read/write device is received by the antenna in the read/write device head or is scanned with an optical reader. This received information is then analyzed by the micro-controller within the read/write device and the identification code is determined. This identification code is then output from the read/write controller via a serial data line which can be fed to a computer. This output of the read/write controller is fed from the read/write controller to a computer system which identifies the particular read/write device, and may combine the identification information with the other information such as information regarding the contents or source of the contents of the vial. Such information could be used to track the contents of the vial from location to location within a lab, or to specifically identify a particular vial when the contents of the vail are in question. Moreover, because of the virtually unlimited number of identification codes which could be programmed into the read/write devices, an unlimited number of vials may be so identified.

Devices specially adapted for opening and closing the microvessels, such as the MICROKAN microvessels, are also provided.

Also provided herein are sensors in which matrices with memories are adapted to detect environmental parameters or

changes or to be implanted in a mammal to detect internal parameters. Sensors containing memories, and coated with polymers and other materials that are responsive to the environment are also provided. In particular, sensors with memories that are coated with electrically conducting polymers are provided.

Also provided are biosensors that are coated with angiogenic factors, such as vascular endothelial growth factors, basic and acid fibroblast growth factors, epidermal growth factors and interleukins are also provided. Such angiogenic coating prevent encapsulation of such devices when implanted in an animal.

Sol-gels containing memories and/or photodetectors and/or photodetectors and memories are also provided. In particular, sol gels containing memories and further adapted for use as sensors are also provided.

DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts combinatorial synthesis of chemical libraries on matrix supports with memories. A, B, C . . . represent the chemical building blocks; a, b, c . . . represent the codes stored in memory that correspond to each of A, B, C, . . . , respectively. S_a, S_b, S_c . . . represent respective signals sent to memory. Alternatively, the matrix supports are OMDs [optical memory devices] that are encoded with symbology associated with information stored in a remote memory, such as a computer. The symbology may be precoded or encoded prior to or during synthesis.

FIG. **2** depicts combinatorial synthesis of peptides on a matrix with memory. Each amino acid has a corresponding code, a, b, c . . . , in the matrix memory, and L represents a Linker between the memory device and the pharmacophore. Again as in FIG. **1**, the matrix supports may be engraved with a code or symbology associated with information stored in a remote memory.

FIG. **3** depicts combinatorial synthesis of oligonucleotides on matrix supports with memories. A, G, T and C represent nucleotides, and a, g, t, and c represent the electronic codes stored in memory that correspond to each of A, G T and C, respectively. The phosphoramidite method of oligonucleotide synthesis is performed by methods known to those of skill in the art [see, e.g., Brown et al. (1991) "Modern machine-aided methods of oligodeoxyribonucleotide synthesis" in Oligonucleotides Analogues EDITOR: Eckstein, Fritz (Ed), IRL, Oxford, UK., pp. 1–24, esp. pp. 4–7]. As in FIGS. **1** and **2**, the matrix may alternatively, or additionally, have symbology engraved thereon.

FIG. **4** depicts generation of a chemical library, such as a library of organic molecules, in which R_1, R_2, R_3 are substituents on selected molecule, such as a pharmacophore monomer, each identified with a different signal, depicted as **1**, **2**, or **3**, from the classes S_1, S_2, S_3, respectively. The circle represents an organic pharmacophore. If R_1–R_3 are the same, and selected from among the same 50 choices, then the complete library contains 50^3=125,000 members. If R_1–R_3 selected from among different sets of choices, then the resulting library has correspondingly more members. Each optical memory device can be encoded with information that represents the R_n added and class [S_n] thereby providing a unique code for each library member. As in FIGS. **1–3**, the matrix may be engraved with symbology, such as a two-dimensional bar code.

FIG. **5** is a block diagram of the data storage means and supporting electrical components of a preferred embodiment.

FIG. **6** is a diagrammatic view of the memory array within the recording device, and the corresponding data stored in the host computer memory.

FIG. **7** is an illustration of an exemplary apparatus for separating the matrix particles with memories for individual exposure to an EM signal.

FIG. **8** is an illustration of a second exemplary embodiment of an apparatus for separating matrix particles for individual exposure to an optical signal.

FIG. **9** is a diagrammatic view of the memory array within the recording device, the corresponding data stored in the host computer memory, and included photodetector with amplifier and gating transistor.

FIG. **10** is a scheme for the synthesis of the 8 member RF encoded combinatorial decameric peptide library described in EXAMPLE 4. All couplings were carried out in DMF at ambient temperature for 1 h [two couplings per amino acid], using PyBOP and EDIA or DIEA. Deprotection conditions: 20% piperidine in DMF, ambient temperature, 30 min; Cleavage conditions: 1,2-ethanedithiol:thioanisole:water:phenol:trifluoroacetic acid [1.5:3:3:4.5:88, w/w], ambient temperature, 1.5 h.

FIG. **11** is a side elevation of a preferred embodiment of a microvessel.

FIG. **12** is a sectional view, with portions cut away, taken along line **12**—**12** of FIG. **11**.

FIG. **13** is a sectional view taken along line **13**—**13** of FIG. **12**.

FIG. **14** is a perspective view of an alternative embodiment of a microvessel, with the end cap separated.

FIG. **15** is a side elevation view of the microvessel of FIG. **14**, with a portion cut away.

FIG. **16** is a sectional view taken along line **16**—**16** of FIG. **15**.

FIG. **17** is a perspective view of an exemplary write/read station.

FIG. **18** is a flow diagram of the operation of the system of FIG. **17**.

FIG. **19** shows fluorescent solid supports: and their application in solid phase synthesis of direct SPA. The supports will include a either an electromagnetically programmable memory, or an optical memory engraved on the surface, such as the 2-D optical bar code provided herein.

FIG. **20** Coded macro "beads" for efficient combinatorial synthesis. As with the supports, these "beads" will include either an electromagnetically programmable memory, or an optical memory engraved on the surface, such as the 2-D optical bar code provided herein.

FIG. **21** Show the preparation and use of a tubular microvessel in which the container is radiation grafted with monomers or otherwise activated for use as a support matrix. As with the supports and "beads", these "beads" will include either an electromagnetically programmable memory, or an optical memory on the surface, such as the 2-D optical bar code provided herein.

FIG. **22** is a perspective view of a first embodiment of an optical memory device;

FIG. **23** is an exploded perspective view of a second embodiment of the optical memory device;

FIG. **24** is a diagrammatic view of the optical write and read for the optical memory devices;

FIG. **25** is a side elevation of a third embodiment of the optical memory device;

FIG. **26** is a side elevation of a fourth embodiment of the optical memory device;

FIG. **27** is a side elevation of a fifth embodiment of the optical memory device;

FIG. 28 is a front elevation of a sixth embodiment of the optical memory device;

FIG. 29 is a front elevation of a seventh embodiment of the optical memory device;

FIG. 30 is a front elevation of an eighth embodiment of the optical memory device;

FIG. 31 is a flow diagram of the image processing sequence for a two-dimensional bar code on an optical memory device; and

FIG. 32 is a diagrammatic view of an exemplary handling system for feeding, reading and distributing the optical memory devices.

FIGS. 33A–E depict the OMDs with optical symbology provided herein. FIG. 33A illustrates an exemplary OMD. FIG. 33B depicts a close-up of a 2-D laser etched bar code that is read by the software described herein that reads the code in two dimensions, horizontally and vertically simultaneously using a camera and pattern recognition software described herein. With reference to the exemplified embodiment [see EXAMPLES], the code in this figure is 0409AA55AA550409. The blacked out and whitened squares represent data units. Etching of the entire 2-D bar code by a CO_2 laser can be accomplished with a resolution below about 0.5 mm. FIG. 33C depicts a split and pool combinatorial synthesis protocol using the OMDs and directed sorting. A, B and C represent building blocks, and the numbers above each OMD represent a 2-D optical bar code [single digits are used merely for exemplification]. FIG. 33D depicts synthesis of a 3×3×3 oligonucleotide library using the OMDs and directed sorting [reaction conditions are described in the EXAMPLES; DMT-X_2, X_3 or X_4 is 5'-O-DMT-2'deoxyadenosine-3'-O-phosphoramidite, 5'-O-DMT-2'deoxycytidine-3'-O-phosphoramidite, 5'-O-DMT-2'deoxyguanosine-3'-O-phosphoramidite; B_2, B_3 or B_4 is adenine, cytosine or guanine. FIG. 33E depicts an oligonucleotide hexamer library using the optical memory device. Each B" refers to a nucleoside base, and the resulting library, where n=6, will contain 4096 unique members [4^6=4096].

FIGS. 34A–D depicts a protocol for radiation grafting of polymers to the inert surfaces to render them suitable for use as matrices. FIG. 34A exemplifies the grafting of a polymer to a tube containing an RF tag, linkage of scintillant to the surface, organic synthesis and then use of the resulting compound linked to the support in an assay. Thus, all steps are performed on the same platform. FIG. 34B also exemplifies a single platform protocol. FIG. 34C depicts the preparation of a tubular devices in which the matrix is the radiation grafted PTFE and the memory is a transponder, such as the BMDS transponder or IDTAG™ transponder [such as a MICROTUBE], described herein; FIG. 34D depicts a small chip [2 mm×2 mm×0.1 mm] encased in a radiation grafted polypropylene or teflon ball [ball or bead or other such geometry] with a screw cap [such as a MICROBALL or MICROBEAD].

FIG. 35 is a perspective view of an alternative embodiment of a exemplary read/write station;

FIG. 36 is a plan view of the read/write station shown in FIG. 35;

FIG. 37 is a cross-sectional view of the read/write station taken along line 37—37 of FIG. 35;

FIG. 38 is a perspective view of a cylindrical tube having a read/write device attached to its lower end;

FIG. 39 is a cross-sectional view of the cylindrical tube and read/write device taken along line 39—39 of FIG. 38;

FIG. 40 is an enlarged cross-sectional view of the read/write device showing the coil antenna, microcircuit, and housing;

FIG. 41 is a perspective view of the microcircuit shown attached to a substrate;

FIG. 42 is a perspective view of the sealing apparatus which is used to create an environmental on the lower end of the housing;

FIG. 43 is a view of a typical program output to the screen showing the decoded identification code, in combination with a graphical representation of the contents of the cylindrical tube.

FIG. 44 is a side elevation view of the capsule sealing tool mounted on a plier type tool;

FIG. 45 is an enlarged sectional view taken on line 47—47 of FIG. 46;

FIG. 46 is a sectional view taken along line 48—48 of FIG. 47, showing the initial cap engagement;

FIG. 47 is a view similar to a portion of FIG. 46, showing the cap fully seated;

FIG. 48 is a view similar to a portion view of FIG. 46, showing ejection of the sealed capsule;

FIG. 49 is a side elevation view of the uncapping tool;

FIG. 50 is a sectional view taken on line 52—52 of FIG. 49, showing the cap separated from the capsule;

FIG. 51 is a perspective view of a monolithic identification tag with the antenna formed on the substrate;

FIG. 52 is a plan view of the monolithic identification tag as shown in FIG. 51, showing generally the outline of the circuitry on the substrate, and the formation of the antenna on encircling that circuitry; and

FIG. 53 is a perspective view of a typical stirring bar with portions of the encapsulation removed to show the positioning of a monolithic identification tag against the metal of the stirring bar.

FIG. 54 is a circuit diagram of the basic components and interconnection of an exemplary sensor with memory;

FIG. 55 is a simple circuit diagram for a temperature sensor;

FIG. 56 is a block diagram of the components of an exemplary implantable glucose sensing system;

FIG. 57 is a cross-section of an exemplary implantable device with logic, power and communication electronics with electrode sensors;

FIG. 58 is a perspective diagrammatic view of the sensor assembly of an exemplary implantable glucose sensing system;

FIG. 59 is a flow diagram the basic control software for the exemplary implantable glucose sensing system;

FIG. 60 is a block diagram of the components of an exemplary intracranial pressure monitor;

FIG. 61 is a diagrammatic view of an exemplary optical intracranial pressure monitor;

FIG. 62 is a diagrammatic view of an alternate optical sensor for use in the intracranial pressure monitor;

FIG. 63 is a diagrammatic view of an exemplary urea sensor located in line with a hemodialysis system;

FIG. 64 is a diagrammatic view of an exemplary embodiment of a "smart" blood bag;

FIG. 65 is a diagrammatic view of an alternate optical sensor for use in a "smart" blood bag; and

FIG. 66 is a diagrammatic view of electrode construction for an alternate embodiment of a glucose sensor.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents and publications referred to herein are, unless noted otherwise, incorporated by reference in their entirety. In the event a definition in this section is not consistent with definitions elsewhere, the defintion set forth in this section will control.

As used herein, a bar codes refers any array optically readable marks of any desired size and shape that are arranged in a reference context or frame of, preferably, although not necessarily, one or more columns and one or more rows. For purposes herein, the bar code refers to any symbology, not necessary "bar" but may include dots, characters or any symbol or symbols.

As used herein, an optical memory refers to the symbology and the surface on which it is engraved or otherwise imprinted or refers to other optical devices. For purposes herein, an optical memory also includes from optical recording media that may be appropriate for use in the recording devices and combinations herein and include, but are not limited to, optical discs, magneto-optical materials, photochromic materials, photoferroelectric materials, and photoconductive electro-optic materials. Optical memories also include memories, such as 2-D and 3-D optical memories that use optics, such as lasers, for writing and/or reading.

As used herein, an optical memory device [OMD] refers to a surface that is encoded with a code, preferably the 2-D bar code provided herein. For use herein, such devices include at least two surfaces, one of which is treated or formed from a matrix material treated to render it suitable for use as a support to which molecules or biological particles are linked, such as in chemical syntheses or as supports in assays, and the other that includes a code that can be optically read and then compared with information in a computer or other memory to interpret its meaning.

As used herein, symbology refers to the code, such as a bar code, that is engraved or imprinted on the OMD. The symbology is any code known or designed by the user. The symbols are associated with information stored in a remote computer or memory or other such device or means. For example, each OMD can be uniquely identified with an encoded symbology. The process steps or additions or manipulations to the associated molecules or biological particles can be recorded in a remote memory and associated with the code.

As used herein, a matrix refers to any solid or semisolid or insoluble support on which a code is to which the memory device and/or the molecule of interest, typically a biological molecule, organic molecule or biospecific ligand is linked or contacted. Typically a matrix is a substrate material having a rigid or semi-rigid surface. In many embodiments, at least one surface of the substrate will be substantially flat, although in some embodiments it may be desirable to physically separate synthesis regions for different polymers with, for example, wells, raised regions, etched trenches, or other such topology. Matrix materials include any materials that are used as affinity matrices or supports for chemical and biological molecule syntheses and analyses, such as, but are not limited to: polystyrene, polycarbonate, polypropylene, nylon, glass, dextran, chitin, sand, pumice, polytetrafluoroethylene, agarose, polysaccharides, dendrimers, buckyballs, polyacrylamide, Kieselguhr-polyacrlamide non-covalent composite, polystyrene-polyacrylamide covalent composite, polystyrene-PEG [polyethyleneglycol] composite, silicon, rubber, and other materials used as supports for solid phase syntheses, affinity separations and purifications, hybridization reactions, immunoassays and other such applications. The matrix herein may be particulate or may be in the form of a continuous surface, such as a microtiter dish or well, a glass slide, a silicon chip with a surface adapted for linking of biological particles or molecules, a nitrocellulose sheet, nylon mesh, or other such materials. When particulate, typically the particles have at least one dimension in the 5–10 mm range or smaller. Such particles, referred collectively herein as "beads", are often, but not necessarily, spherical. Such reference, however, does not constrain the geometry of the matrix, which may be any shape, including random shapes, needles, fibers, elongated, etc. The "beads" may include additional components, such as magnetic or paramagnetic particles [see, e.g., Dyna beads (Dynal, Oslo, Norway)] for separation using magnets, fluophores and other scintillants, as long as the additional components do not interfere with chemical reactions, data entry or retrieval from the memory.

Significantly, it is noted, however, that many surfaces, such as glass, require modification to render them suitable for use as supports. Any such surface must be treated to render it suitable for chemical syntheses or for adsorption of biological particles. Chemical syntheses require a support that not only has the proper surface characteristics (organic solvent wettability, chemical kinetics, etc.), but that also has a high density of functional groups. An untreated glass surface contains only a very small amount [less than 1 nmol/sq. mm] of hydroxy groups. It is also very hydrophilic and not very suitable for reactions in organic media. Therefore, the glass surface has to be modified to achieve high functional group density (–>10 nmol/mm^2) and proper hydrophobicity. Thus, as used herein, matrix refers to materials that have been so-treated. Therefore, a transponder in which the memory device is encased in a glass capsule for instance is not usable as is, but must be treated, either by coating at least one surface with a polymer, such as by grafting, derivatizing or otherwise activating the surface.

As used herein, scintillants include, 2,5-diphenyloxazole [PPO], anthracene, 2-(4'-tert-butylphenyl)-5-(4"-biphenyl)-1,3,4-oxadiazole [butyl-PBD]; 1-phenyl-3-mesityl-2-pyrazoline [PMP], with or without frequency shifters, such as 1,4,-bis[5-phenyl(oxazolyl)benzene] [POPOP]; p-bis-o-methylstyrylbenzene [bis-MSB]. Combinations of these fluors, such as PPO and POPOP or PPO and bis-MSB, in suitable solvents, such as benzyltoluene [see, e.g., U.S. Pat. No. 5,410,155], are referred to as scintillation cocktails.

As used herein a luminescent moiety refers to a scintiilant or fluophor used in scintillation proximity assays or in non-radioactive energy transfer assays, such as HTRF assays.

As used herein, fluorescent resonance energy transfer [FRET] is an art-recognized term meaning that one fluorophore [the acceptor] can be promoted to an excited electronic state through quantum mechanical coupling with and receipt of energy from an electronically excited second fluorophore [the donor]. This transfer of energy results in a decrease in visible fluorescence emission by the donor and an increase in fluorescent energy emission by the acceptor. Significant energy transfer can only occur when the donor and acceptor are sufficiently closely positioned since the efficiency of energy transfer is highly dependent upon the distance between donor and acceptor fluorophores.

As used herein, matrix particles refer to matrix materials that are in the form of discrete particles. The particles have any shape and dimensions, but typically have at least one dimension that is 100 mm or less, preferably 50 mm or less, more preferably 10 mm or less, and typically have a size that is 100 mm^3 or less, preferably 50 mm^3 or less, more preferably mm^3 or less, and most preferably 1 mm^3 or less. The matrices may also be continuous surfaces, such as microtiter plates [e.g., plates made from polystyrene or polycarbonate or derivatives thereof commercially available from Perkin Elmer Cetus and numerous other sources, and Covalink trays [Nunc], microtiter plate lids or a test tube, such as a 1 ml Eppendorf tube or smaller versions, such as 500 μl, 200 μl or smaller. Matrices that are in the form of containers refers to containers, such as test tubes and microplates and vials that are typically used for solid phase syntheses of combinatorial libraries or as pouches, vessels, bags, and microvessels for screening and diagnostic assays or as containers for samples, such as patient samples. Thus, a container used for chemical syntheses refers to a container that typically has a volume of about 1 liter, generally 100 ml, and more often 10 ml or less, 5 ml or less, preferably 1 ml or less, and as small as about 50 μl–500 μl, such as 100 μl or 250 μl or 200 μl. This also refers to multi-well plates, such as microtiter plates [96 well, 384 well, 1536 well or other higher density format]. Such microplate will typically contain a memory device in, on, or otherwise in contact with in each of a plurality of wells.

As used herein, a matrix with a memory refers to a combination of a matrix with a miniature recording device that stores multiple bits of data by which the matrix may be identified, preferably in a non-volatile memory that can be written to and read from by transmission of electromagnetic radiation from a remote host, such as a computer. By miniature is meant of a size less than about 10–20 mm^3 [or 10–20 mm in the largest dimension]. Preferred memory devices or data storage units are miniature and are preferably smaller than 10–20 mm^3 [or 10–20 mm in its largest dimension] dimension, more preferably less than 5 mm^3, most preferably about 1 mm^3 or smaller. Alternatively, the memory may be fabricated as part of the matrix material or may be a chemical or biological-based memory means, such as those described herein, including the rhodopsin based memories and 3-D optical memories based on photochromic materials [see, e.g., U.S. Pat. Nos. 5,268,862, 5,130,362, 5,325,324; see, also, Dvornikov et al. (1996) *Opt. Commun.* 128:205–210;

Dvornikov et al. (1996) *Res. Chem. Intermed.* 22:115–28; Dvornikov et al. (1994) *Proc. SPIE-Int. Soc. Opt. Eng.* 2297:447–51; Dvornikov et al. (1994) *Mol. Cryst. Lig. Cryst. Sci. Technol., Sect. A* 246:379–88; Dvornikov et al. (1994) *J. Phys. Chem.* 98:6746–52; Ford et al. (1993) *Proc. SPIE-Int. Soc. Opt.* 2026:604–613; Ford et al. *Proc. SPIE-Int. Soc. Opt. Eng.* 1853:5–13; Malkin et al. *Res. Chem. Intermed.* 19:159–89; Dvornikov et al. (1993) *Proc. SPIE-Int. Soc. Opt. Eng.* 1852:243–52; Dvornikov et al. (1992) *Proc. SPIE-Int. Soc. Opt. Eng.* 1662:197–204; Prasad et al. (1996) *Mater. Res. Soc. Symp. Proc.* 413:203–213].

As used herein, a microreactor refers to combinations of matrices with memories with associated, such as linked or proximate, biological particles or molecules. It is produced, for example, when the molecule is linked thereto or synthesized thereon. It is then used in subsequent protocols, such as immunoassays and scintillation proximity assays.

As used herein, a combination herein called a microvessel [e.g., a microvessel such as those designated presently designated a MICROKAN™] refers to a combination in which a single device for more than one device] and a plurality of particles are sealed in a porous or semi-permeable inert material, such as polytetrafluoroethylene or polypropylene or membrane that is permeable to the components of the medium, but retains the particles and memory, or are sealed in a small closable container that has at least one dimension that is porous or semi-permeable. Typically such microvessels, which preferably have at least one end that can be opened and sealed or closed tightly, has a volume of about 200–500 mm^3, with preferred dimensions of about 1–10 mm in diameter and 5 to 20 mm in height, more preferably about 5 mm by 15 mm. The porous wall should be non-collapsible with a pore size in the range of 70 μM to about 100 μM, but can be selected to be semi-permeable for selected components of the reaction medium.

As used herein, a memory is a data storage unit [or medium] with programmable memory, preferably a non-volatile memory; or alternatively is a symbology on a surface, such as a bar code, whose identity and as for which associate information is stored in a remote memory, such as a computer memory.

As used herein, programming refers to the process by which data or information is entered and stored in a memory. A memory that is programmed is a memory that contains retrievable information.

As used herein, remotely programmable, means that the memory can be programmed without direct physical or electrical contact or can be programmed from a distance, typically at least about 10 mm, although shorter distances may also be used, such as instances in which the information comes from surface or proximal reactions or from an adjacent memory or in instances, such as embodiments in which the memories are very close to each other, as in microtiter plate wells or in an array.

As used herein, a recording device [or memory device] is an apparatus that includes the data storage unit with programmable memory, and, if necessary, means for receiving information and for transmitting information that has been recorded. It includes any means needed or used for writing to and reading from the memory. The recording devices intended for use herein, are miniature devices that preferably are smaller than 10–20 mm^3 [or 10–20 mm in their largest dimension], and more preferably are closer in size to 1 mm^3 or smaller that contain at least one such memory and means for receiving and transmitting data to and from the memory. The data storage device also includes optical memories, such as bar codes, on devices such as OMDs.

As used herein, a data storage unit with programmable memory includes any data storage means having the ability to record multiple discrete bits of data, which discrete bits of data may be individually accessed [read] after one or more recording operations. Thus, a matrix with memory is a combination of a matrix material with a data storage unit.

As used herein, programmable means capable of storing unique data points. Addressable means having unique locations that may be selected for storing the unique data points.

As used herein, reaction verifying and reaction detecting are interchangeable and refer to the combination that also includes elements that detect occurrence of a reaction or event of interest between the associated molecule or biological particle and its environment [i.e., detects occurrence of a reaction, such as ligand binding, by virtue of emission of EM upon reaction or a change in pH or temperature or other parameter].

As used herein, a host computer or decoder/encoder instrument is an instrument that has been programmed with

or includes information [i.e., a key] specifying the code used to encode the memory devices. This instrument or one linked thereto transmits the information and signals to the recording device and it, or another instrument, receives the information transmitted from the recording device upon receipt of the appropriate signal. This instrument thus creates the appropriate signal to transmit to the recording device and can interpret transmitted signals. For example, if a "1" is stored at position 1,1 in the memory of the recording device means, upon receipt of this information, this instrument or computer can determine that this means the linked molecule is, for example, a peptide containing alanine at the N-terminus, an organic group, organic molecule, oligonucleotide, or whatever this information has been predetermined to mean. Alternatively, the information sent to and transmitted from the recording device can be encoded into the appropriate form by a person.

As used herein, an electromagnetic tag is a recording device that has a memory that contains unique data points that correspond to information that identifies molecules or biological particles linked to, directly or indirectly, in physical contact with or in proximity [or associated with] to the device. Thus, electromagnetic tagging is the process by which identifying or tracking information is transmitted [by any means and to any recording device memory, including optical and magnetic storage media] to the recording device.

As used herein, proximity means within a very short distance, generally less than 0.5 inch, typically less than 0.2 inches. In particular, stating that the matrix material and memory, or the biological particle or molecule and matrix with memory are in proximity means that, they are at least or at least were in the same reaction vessel or, if the memory is removed from the reaction vessel, the identity of the vessel containing the molecules or biological particles with which the memory was proximate or linked is tracked or otherwise known.

As used herein, associated with means that the memory must remain in proximity to the molecule or biological particle or must in some manner be traceable to the molecule or biological particle. For example, if a molecule is cleaved from the support with memory, the memory must in some manner be identified as having been linked to the cleaved molecule. Thus, a molecule or biological particle that had been linked to or in proximity to a matrix with memory is associated with the matrix or memory if it can be identified by querying the memory.

As used herein, antifuse refers to an electrical device that is initially an open circuit that becomes a closed circuit during programming, thereby providing for non-volatile memory means and, when accompanied by appropriate transceiver and rectification circuitry, permitting remote programming and, hence identification. In practice, an antifuse is a substantially nonconductive structure that is capable of becoming substantially conductive upon application of a predetermined voltage, which exceeds a threshold voltage. An antifuse memory does not require a constant voltage source for refreshing the memory and, therefore, may be incorporated in a passive device. Other memories that may be used include, but are not limited to: EEPROMS, DRAMS and flash memories.

As used herein, flash memory is memory that retains information when power is removed [see, e.g., U.S. Pat. No. 5,452,311, U.S. Pat. No. 5,452,251 and U.S. Pat. No. 5,449,941]. Flash memory can be rewritten by electrically and collectively erasing the stored data, and then by programming.

As used herein, passive device refers to an electrical device which does not have its own voltage source and relies upon a transmitted signal to provide voltage for operation.

As used herein, electromagnetic [EM] radiation refers to radiation understood by skilled artisans to be EM radiation and includes, but is not limited to radio frequency [RF], infrared [IR], visible, ultraviolet [UV], radiation, sonic waves, X-rays, and laser light.

As used herein, information identifying or tracking a biological particle or molecule, refers to any information that identifies the molecule or biological particle, such as, but not limited to the identity particle [i.e. its chemical formula or name], its sequence, its type, its class, its purity, its properties, such as its binding affinity for a particular ligand. Tracking means the ability to follow a molecule or biological particle through synthesis and/or process steps. The memory devices herein store unique indicators that represent any of this information.

As used herein, combinatorial chemistry is a synthetic strategy that produces diverse, usually large, chemical libraries. It is the systematic and repetitive, covalent connection of a set, the basis set, of different monomeric building blocks of varying structure to each other to produce an array of diverse molecules [see, e.g., Gallop et al. (1994) *J. Medicinal Chemistry* 37:1233–1251]. It also encompasses other chemical modifications, such as cyclizations, eliminations, cleavages, etc., that are carried in manner that generates permutations and thereby collections of diverse molecules.

As used herein, a biological particle refers to a virus, such as a viral vector or viral capsid with or without packaged nucleic acid, phage, including a phage vector or phage capsid, with or without encapsulated nucleotide acid, a single cell, including eukaryotic and prokaryotic cells or fragments thereof, a liposome or micellar agent or other packaging particle, and other such biological materials.

As used herein, the molecules in the combinations include any molecule, including nucleic acids, amino acids, other biopolymers, and other organic molecules, including peptidomimetics and monomers or polymers of small organic molecular constituents of non-peptidic libraries, that may be identified by the methods here and/or synthesized on matrices with memories as described herein.

As used herein, the term "bio-oligomer" refers to a biopolymer of less than about 100 subunits. A bio-oligomer includes, but is not limited to, a peptide, i.e., containing amino acid subunits, an oligonucleotide, i.e., containing nucleoside subunits, a peptide-oligonucleotide chimera, peptidomimetic, and a polysaccharide.

As used herein, the term "sequences of random monomer subunits" refers to polymers or oligomers containing sequences of monomers in which any monomer subunit may precede or follow any other monomer subunit.

As used herein, the term "library" refers to a collection of substantially random compounds or biological particles expressing random peptides or proteins or to a collection of diverse compounds. Of particular interest are bio-oligomers, biopolymers, or diverse organic compounds or a set of compounds prepared from monomers based on a selected pharmacophore.

As used herein, an analyte is any substance that is analyzed or assayed in the reaction of interest. Thus, analytes include the substrates, products and intermediates in the reaction, as well as the enzymes and cofactors.

As used herein, multianalyte analysis is the ability to measure many analytes in a single specimen or to perform

multiple tests from a single specimen. The methods and combinations herein provide means to identify or track individual analytes from among a mixture of such analytes.

As used herein, a fluophore or a fluor is a molecule that readily fluoresces; it is a molecule that emits light following interaction with radiation. The process of fluorescence refers to emission of a photon by a molecule in an excited singlet state. For scintillation assays, combinations of fluors are typically used. A primary fluor that emits light following interaction with radiation and a secondary fluor that shifts the wavelength emitted by the primary fluor to a higher more efficiently detected wavelength.

As used herein, a peptidomimetic is a compound that mimics the conformation and certain stereochemical features of the biologically active form of a particular peptide. In general, peptidomimetics are designed to mimic certain desirable properties of a compound but not the undesirable features, such as flexibility leading to a loss of the biologically active conformation and bond breakdown. For example, methylenethio bioisostere [CH_2S] has been used as an amide replacement in enkephalin analogs [see, e.g., Spatola, A. F. *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins* [Weinstein, B, Ed., Vol. 7, pp. 267–357, Marcel Dekker, New York (1983); and Szelke et al. (1983) *In Peptides: Structure and Function, Proceedings of the Eighth American Peptide Symposium*, Hruby and Rich, Eds., pp. 579–582, Pierce Chemical Co., Rockford, Ill.].

As used herein, complete coupling means that the coupling reaction is driven substantially to completion despite or regardless of the differences in the coupling rates of individual components of the reaction, such as amino acids In addition, the amino acids, or whatever is being coupled, are coupled to substantially all available coupling sites on the solid phase support so that each solid phase support will contain essentially only one species of peptide.

As used herein, the biological activity or bioactivity of a particular compound includes any activity induced, potentiated or influenced by the compound in vivo or in vitro. It also includes the abilities, such as the ability of certain molecules to bind to particular receptors and to induce [or modulate] a functional response. It may be assessed by in vivo assays or by in vitro assays, such as those exemplified herein.

As used herein, pharmaceutically acceptable salts, esters or other derivatives of the compounds include any salts, esters or derivatives that may be readily prepared by those of skill in this art using known methods for such derivatization and that produce compounds that may be administered to animals or humans without substantial toxic effects and that either are pharmaceutically active or are prodrugs. For example, hydroxy groups can be esterified or etherified.

As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography [TLC], mass spectrometry [MS], size exclusion chromatography, gel electrophoresis, particularly agarose and polyacrylamide gel electrophoresis [PAGE] and high performance liquid chromatography [HPLC], used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound may, however, be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound.

As used herein, adequately pure or "pure" per se means sufficiently pure for the intended use of the adequately pure compound.

As used herein, biological activity refers to the in vivo activities of a compound or physiological responses that result upon in vivo administration of a compound, composition or other mixture. Biological activity, thus, encompasses therapeutic effects and pharmaceutical activity of such compounds, compositions and mixtures.

As used herein, a prodrug is a compound that, upon in vivo administration, is metabolized or otherwise converted to the biologically, pharmaceutically or therapeutically active form of the compound. To produce a prodrug, the pharmaceutically active compound is modified such that the active compound will be regenerated by metabolic processes. The prodrug may be designed to alter the metabolic stability or the transport characteristics of a drug, to mask side effects or toxicity, to improve the flavor of a drug or to alter other characteristics or properties of a drug. By virtue of knowledge of pharmacodynamic processes and drug metabolism in vivo, those of skill in this art, once a pharmaceutically active compound is known, can design prodrugs of the compound [see, e.g., Nogrady (1985) *Medicinal Chemistry A Biochemical Aproach*, Oxford University Press, New York, pages 388–392].

As used herein, amino acids refer to the naturally-occurring amino acids and any other non-naturally occurring amino acids, and also the corresponding D-isomers. It is also understood that certain amino acids may be replaced by substantially equivalent non-naturally occurring variants thereof, such as D-Nva, D-Nle, D-Alle, and others listed with the abbreviations below or known to those of skill in this art.

As used herein, hydrophobic amino acids include Ala, Val, Leu, Ile, Pro, Phe, Trp, and Met, the non-naturally occurring amino acids and the corresponding D isomers of the hydrophobic amino acids, that have similar hydrophobic properties; the polar amino acids include Gly, Ser, Thr, Cys, Tyr, Asn, Gln, the non-naturally occurring amino acids and the corresponding D isomers of the polar amino acids, that have similar properties, the charged amino acids include Asp, Glu, Lys, Arg, His, the non-naturally occurring amino acids and the corresponding D isomers of these amino acids.

As used herein, Southern, Northern, Western and dot blot procedures refer to those in which DNA, RNA and protein patterns, respectively, are transferred for example, from agarose gels, polyacrylamide gels or other suitable medium that constricts convective motion of molecules, to nitrocellulose membranes or other suitable medium for hybridization or antibody or antigen binding are well known to those of skill in this art [see, e.g., Southern (1975) *J. Mol. Biol.* 98:503–517; Ketner et al. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73:1102–1106; Towbin et al. (1979) *Proc. Natl. Acad. Sci. U.S.A.* 76:4350].

As used herein, a receptor refers to a molecule that has an affinity for a given ligand. Receptors may be naturally-occurring or synthetic molecules. Receptors may also be referred to in the art as anti-ligands. As used herein, the terms, receptor and anti-ligand are interchangeable. Receptors can be used in their unaltered state or as aggregates with other species. Receptors may be attached, covalently or noncovalently, or in physical contact with, to a binding member, either directly or indirectly via a specific binding substance or linker. Examples of receptors, include, but are

not limited to: antibodies, cell membrane receptors surface receptors and internalizing receptors, monoclonal antibodies and antisera reactive with specific antigenic determinants [such as on viruses, cells, or other materials], drugs, polynucleotides, nucleic acids, peptides, cofactors, lectins, sugars, polysaccharides, cells, cellular membranes, and organelles.

Examples of receptors and applications using such receptors, include but are not restricted to:

a) enzymes: specific transport proteins or enzymes essential to survival of microorganisms, which could serve as targets for antibiotic [ligand] selection;

b) antibodies: identification of a ligand-binding site on the antibody molecule that combines with the epitope of an antigen of interest may be investigated; determination of a sequence that mimics an antigenic epitope may lead to the development of vaccines of which the immunogen is based on one or more of such sequences or lead to the development of related diagnostic agents or compounds useful in therapeutic treatments such as for auto-immune diseases

c) nucleic acids: identification of ligand, such as protein or RNA, binding sites;

d) catalytic polypeptides: polymers, preferably polypeptides, that are capable of promoting a chemical reaction involving the conversion of one or more reactants to one or more products; such polypeptides generally include a binding site specific for at least one reactant or reaction intermediate and an active functionality proximate to the binding site, in which the functionality is capable of chemically modifying the bound reactant [see, e.g., U.S. Pat. No. 5,215,899];

e) hormone receptors: determination of the ligands that bind with high affinity to a receptor is useful in the development of hormone replacement therapies; for example, identification of ligands that bind to such receptors may lead to the development of drugs to control blood pressure; and

f) opiate receptors: determination of ligands that bind to the opiate receptors in the brain is useful in the development of less-addictive replacements for morphine and related drugs.

As used herein, antibody includes antibody fragments, such as Fab fragments, which are composed of a light chain and the variable region of a heavy chain.

As used herein, complementary refers to the topological compatibility or matching together of interacting surfaces of a ligand molecule and its receptor. Thus, the receptor and its ligand can be described as complementary, and furthermore, the contact surface characteristics are complementary to each other.

As used herein, a ligand-receptor pair or complex formed when two macromolecules have combined through molecular recognition to form a complex.

As used herein, an epitope refers to a portion of an antigen molecule that is delineated by the area of interaction with the subclass of receptors known as antibodies.

As used herein, a ligand is a molecule that is specifically recognized by a particular receptor. Examples of ligands, include, but are not limited to, agonists and antagonists for cell membrane receptors, toxins and venoms, viral epitopes, hormones [e.g., steroids], hormone receptors, opiates, peptides, enzymes, enzyme substrates, cofactors, drugs, lectins, sugars, oligonucleotides, nucleic acids, oligosaccharides, proteins, and monoclonal antibodies.

As used herein, a sensor is a device or apparatus that monitors external (or internal) parameters (i.e., conditions), such as ion concentrations, pH, temperatures, and events. Internal parameters refer to conditions, concentrations, such as electrolyte and glucose concentration, in an animal. Biosensors are sensors that detect biological species. Sensors encompass devices that rely on electrochemical, optical, biological and other such means to monitor the environment.

As used herein, multiplexing refers to performing a series of synthetic and processing steps and/or assaying steps on the same platform [i.e. solid support or matrix] or coupled together as part of the same automated coupled protocol, including one or more of the following, synthesis, preferably accompanied by writing to the linked memories to identify linked compounds, screening, including using protocols with matrices with memories, and compound identification by querying the memories of matrices associated with the selected compounds. Thus, the platform refers system in which all manipulations are performed. In general it means that several protocols are coupled and performed sequentially or simultaneously.

As used herein, a platform refers to the instrumentation or devices in which on which a reaction or series of reactions is(are) performed.

As used herein a protecting group refers to a material that is chemically bound to a monomer unit that may be removed upon selective exposure to an activator such as electromagnetic radiation and, especially ultraviolet and visible light, or that may be selectively cleaved. Examples of protecting groups include, but are not limited to: those containing nitropiperonyl, pyrenylmethoxy-carbonyl, nitroveratryl, nitrobenzyl, dimethyl dimethoxybenzyl, 5-bromo-7-nitroindolinyl, o-hydroxy- alpha -methyl cinnamoyl, and 2-oxymethylene anthraquinone.

Also protected amino acids are readily available to those of skill in this art. For example, Fmoc and Boc protected amino acids can be obtained from Fluka, Bachem, Advanced Chemtech, Sigma, Cambridge Research Biochemical, Bachem, or Peninsula Labs or other chemical companies familiar to those who practice this art.

As used herein, the abbreviations for amino acids and protective groups are in accord with their common usage and the IUPAC-IUB Commission on Biochemical Nomenclature [see, (1972) *Biochem.* 11: 942–944]. Each naturally occurring L-amino acid is identified by the standard three letter code or the standard three letter code with or without the prefix "L-"; the prefix "D-" indicates that the stereoisomeric form of the amino acid is D. For example, as used herein, Fmoc is 9-fluorenylmethoxycarbonyl; BOP is benzotriazol-1-yloxy-tris(dimethylamino) phosphonium hexafluorophosphate, DCC is dicyclohexyl-carbodiimide; DDZ is dimethoxydimethylbenzyloxy; DMT is dimethoxytrityl; FMOC is fluorenylmethyloxycarbonyl; HBTU is 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium; hexafluorophosphate NV is nitroveratryl; NVOC is 6-nitroveratryloxycarbonyl and other photoremovable groups; TFA is trifluoroacetic acid; DMF for N,N-dimethylformamide; Boc is tert-butoxycarbonyl; ACN is acetonitrile, TFA for trifluoroacetic acid; HF for hydrogen fluoride; HFIP for hexafluoroisopropanol; HPLC for high performance liquid chromatography; FAB-MS for fast atom bombardment mass spectrometry; DCM is dichloromethane, Bom is benzyloxymethyl; Pd/C is palladium catalyst on activated charcoal; DIC is diisopropylcarbodiimide; DCC is N,N'-dicyclohexylcarbodiimide; [For] is formyl; PyBop is benzotriazol-1-yl-oxy-trispyrrolidino-phosphonium hexafluoro-phosphate; POPOP is 1,4,-bis[5-phenyl (oxazolyl)benzenel; PPO is 2,5-diphenyloxazole; butyl-PBD is [2-(4'-tert-butylphenyl)-5-(4"-biphenyl)-1,3,4-

oxadiazole]; PMP is (1-phenyl-3-mesityl-2-pyrazoline) DIEA is diisopropylethyl-amine; EDIA is ethyidiisopropylethylamine; NMP is N-methylpyrrolidone; NV is nitroveratryl PAL is pyridylalanine; HATU is O(7-azabenzotriaol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; TFA is trifluoracetic acid, THF is tetrahydrofuran; and EDT is 1,2-ethanedithiol.

A. Matrices

For purposes herein matrices refer to supports used to retain molecules and biological particles, such as for chemical synthesis and to containers, such as microplates and test tubes. Matrices used for supports will be derivatized or otherwise suitable for retaining molecules or biological particles. Containers will either be derivatized or otherwise suitable for retaining molecules or biological particles or will be suitable for containing molecules and biological particles. In the embodiments of interest herein, the matrices are engraved with an optical bar code, include a memory and are associated with a sensor or are associated with containers, laboratory equipment and other such devices.

Matrices, which are generally insoluble materials used to immobilize ligands and other molecules, have application in many chemical syntheses and separations. Matrices are used in affinity chromatography, in the immobilization of biologically active materials, and during chemical syntheses of biomolecules, including proteins, amino acids and other organic molecules and polymers. The preparation of and use of matrices is well known to those of skill in this art; there are many such materials and preparations thereof known. For example, naturally-occurring matrix materials, such as agarose and cellulose, may be isolated from their respective sources, and processed according to known protocols, and synthetic materials may be prepared in accord with known protocols.

Matrices include any material that can act as a support matrix for attachment of the molecules or biological particles of interest and can be in contact with or proximity to or associated with, preferably encasing or coating, the data storage device with programmable memory. Any matrix composed of material that is compatible with and upon or in which chemical syntheses are performed, including biocompatible polymers, is suitable for use herein. The matrix material should be selected so that it does not interfere with the chemistry or biological reaction of interest during the time which the molecule or particle is linked to, or in proximity therewith [see, e.g., U.S. Pat. No. 4,006,403]. These matrices, thus include any material to which the data storage device with memory can be attached, placed in proximity thereof, impregnated, encased or otherwise connected, linked or physically contacted. Such materials are known to those of skill in this art, and include those that are used as a support matrix. These materials include, but are not limited to, inorganics, natural polymers, and synthetic polymers, including, but are not limited to: cellulose, cellulose derivatives, acrylic resins, glass that is derivatized to render it suitable for use a support, silica gels, polystyrene, gelatin, polyvinyl pyrrolidone, co-polymers of vinyl and acrylamide, polystyrene cross-linked with divinylbenzene or the like [see, Merrifield (1964) Biochemistry 3:1385–1390], polyacrylamides, latex gels, polystyrene, dextran, polyacrylamides, rubber, silicon, plastics, nitrocellulose, celluloses, natural sponges, and many others. It is understood that the matrix materials contemplated are those that are suitable for use a s support matrix for retaining molecules or biological particles during syntheses or reactions.

Among the preferred matrices are polymeric beads, such as the TENTAGEL™ resins and derivatives thereof [sold by Rapp Polymere, Tubingen, Germany; see, U.S. Pat. No. 4,908,405 and U.S. Pat. No. 5,292,814; see, also Butz et al. (1994) *Peptide Res.* 7:20–23; Kleine et al. (1994) *Immunobiol.* 190:53–66; see, also Piskin et al. (1994), Chapter 18 "Nondegradable and Biodegradable Polymeric Particles" in *Diagnostic Biosensor Polymers*, ACS Symp. Series 556, Usmani et al. Eds, American Chemical Society, Washington, DC], which are designed for solid phase chemistry and for affinity separations and purifications. See, also Bayer et al. (1994) in *Pept.: Chem., Struct. Biol., Proc. Am. Pept. Symp.*, 13*th*; Hodges, et al. eds., pp.156–158; Zhang et al. (1993) *Pept.* 1992, *Proc. Eur. Pent. Symp.*, 22*nd*, Schneider, et al., eds. pp. 432–433; llg et al. (1994) *Macromolecules*, pp. 2778–83; Zeppezauer et al. (1993) *Z. Naturforsch., B: Chem. Sci.* 48:1801–1806; Rapp et al. (1992) *Pept. Chem.* 1992, *Proc. Jpn. Symp.*, 2*nd*, Yanaihara, ed., pp. 7–10; Nokihara et al. (1993) *Shimadzu Hyoron* 50:25–31; Wright et al. (1993) *Tetrahedron Lett.* 34:3373–3376; Bayer et al. (1992) *Poly(Ethylene Glycol) Chem.* Harris, ed., pp. 325–45; Rapp et al. (1990) *Innovation Perspect. Solid Phase Synth. Collect. Pap., Int. Symp.*, 1*st*, Epton, ed., pp. 205–10; Rapp et al. (1992) *Pept.: Chem. Biol., Proc. Am. Pept. Symp.*, 12*th*, Smith et al., eds., pp. 529–530; Rapp et al. (1989) *Pept., Proc. Eur. Pept. Symp.*, 20*th*, Jung et al., ed., pp. 199–201; Bayer et al. (1986) *Chem. pept. Proteins* 3: 3–8; Bayer et al. (1983) *Pept.: Struct. Funct., Proc. Am. pept. Symp.*, 8*th*, Hruby et al. eds., pp. 87–90 for descriptions of preparation of such beads and use thereof in synthetic chemistry. Matrices that are also contemplated for use herein include fluophore-containing or -impregnated matrices, such as microplates and beads [commercially available, for example, from Amersham, Arlington Heights, Ill; plastic scintillation beads from NE (Nuclear Technology, Inc., San Carlos, Calif.), Packard, Meriden, Conn.]. It is understood that these commercially available materials will be modified by combining them with memories, such as by methods described herein.

The matrix may also be a relatively inert polymer, which can be grafted by ionizing radiation [see, e.g., FIG. 21, which depicts a particular embodiment] to permit attachment of a coating of polystyrene or other such polymer that can be derivatized and used as a support. Radiation grafting of monomers allows a diversity of surface characteristics to be generated on plasmid supports [see, e.g., Maeji et al. (1994) *Reactive Polymers* 22:203–212; and Berg et al. (1989) *J. Am. Chem. Soc.* 111 :8024–8026]. For example, radiolytic grafting of monomers, such as vinyl monomers, or mixtures of monomers, to polymers, such as polyethylene and polypropylene, produce composites that have a wide variety of surface characteristics. These methods have been used to graft polymers to insoluble supports for synthesis of peptides and other molecules, and are of particular interest herein. The recording devices, which are often coated with a plastic or other insert material, can be treated with ionizing radiation so that selected monomers can be grafted to render the surface suitable for chemical syntheses.

Where the matrix particles are macroscopic in size, such as about at least 1 mm in at least one dimension, such bead or matrix particle or continuous matrix may contain one or more memories. Where the matrix particles are smaller, such as NE particles [PVT-based plastic scintillator microsphere], which are about 1 to 10 μm in diameter, more than one such particle will generally be associated with one memory. Also, the "bead" or plate or container may include additional material, such as scintillant or a fluophore impregnated therein. In preferred embodiments, the solid phase chemistry and subsequent assaying may be performed on the same

bead or matrix with memory combination. All procedures, including synthesis on the bead and assaying and analysis, can be automated.

The matrices are typically insoluble substrates that are solid, porous, deformable, or hard, and have any required structure and geometry, including, but not limited to: beads, pellets, disks, capillaries, hollow fibers, needles, solid fibers, random shapes, thin films and membranes. Typically, when the matrix is particulate, the particles are at least about 10–2000 μM, but may be smaller, particularly for use in embodiments in which more than one particle is in proximity to a memory. For purposes herein, the support material will typically encase or be in contact with the data storage device, and, thus, will desirably have at least one dimension on the order of 1 mm [1000 μM] or more, although smaller particles may be contacted with the data storage devices, particularly in embodiments in which more than one matrix particle is associated, linked or in proximity to one memory or matrix with memory, such as the microvessels [see, e.g., FIGS. 11–16]. Each memory will be in associated with, in contact with or proximity to at least one matrix particle, and may be in contact with more than one. As smaller semiconductor and electronic or optical devices become available, the capacity of the memory can be increased and/or the size of the particles can be decreased. For example, presently, 0.5 micron semiconductor devices are available. Integrated circuits 0.25-micron in size have been described and are being developed using a technology called the Complementary Metal Oxide-Semiconductor process (see, e.g., Investor's Business Daily May 30, 1995).

Also of interest herein, are devices that are prepared by inserting the recording device into a "tube" [see, e.g., FIG. 21] or encasing them in an inert material [with respect to the media in which the device will be in contact]. This material is fabricated from a plastic or other inert material. Preferably prior to introducing [and preferably sealing] the recording device inside, the tube or encasing material is treated with ionizing radiation to render the surface suitable for grafting selected monomers, such as styrene [see, e.g., Maeji et al. (1994) *Reactive Polymers* 22:203–212; Ang et al. in Chapter 10: Application of Radiation Grafting in Reagent Insolubilization, pp 223–247; and Berg et al. (1989) *J. Am. Chem. Soc.* 111:8024–8026].

Recording device(s) is(are) introduced inside the material or the material is wrapped around the device and the resulting matrix with memory "tubes" [MICROTUBES™, see; FIG. 21] are used for chemical synthesis or linkage of selected molecules or biological particles. These "tubes" are preferably synthesized from an inert resin, such as a polypropylene resin [e.g., a Moplen resin, V29G PP resin from Montell, Newark Del., a distributor for Himont, Italy]. Any inert matrix that can then be functionalized or to which derivatizable monomers can be grafted is suitable. Preferably herein, polypropylene tubes are grafted and then formed into tubes or other suitable shape and the recording device inserted inside. These tubes [MICROTUBES™] with grafted monomers are then used as synthesis, and/or for assays or for multiplexed processes, including synthesis and assays or other multistep procedures.

Such tubes may also have snap on or screw tops so that the memory device or chip is removable. For example, they may be conical tubes like Eppendorf tubes, with a snap on top, preferably a flat top. The tubes will be of a size to accommodate a memory device and thus may be as small as about 2 mm×2 mm×0.1 to hold the small 2 mm×2 mm×0.1 mm device described herein. They will be fabricated from polypropylene or other suitable material and radiation grafted, see above, and Examples, below, preferably prior to introduction of the memory device.

Solid tubular embodiments, such as the MICROTUBE microreactors made of tubes for other geometry] have been coated or grafted with suitable materials are used as a solid support for any other methods disclosed herein, including organic syntheses and assays. Fluorophores, scintillants and other such compounds may also be incorporated into the surface or linked thereto [see, EXAMPLES below]. These tubes include those that contain the memory encased either permanently or removably or that include an imprinted symbology.

Briefly, for radiation-induced graft copolymerization, for example, of styrene to polypropylene (PP), polyethylene (PE) and teflon (PTFE) tubes, the diameter of the tube can be any desired size, with 0.1 mm to 20 mm presently preferred and 2 mm to 5 mm more preferred. It has been found that dilution of styrene with methanol enhances the rate of grafting, thereby permitting use of PTFE tubes. Dilutions, which can be determined empirically for each material, from 5% to 70% have been tested. PTFE and PE tubes have the highest styrene grafting at a 50% dilution, and polypropylene tubes have the best performance when grafted at a 35% dilution. To effect grafting the polymer tubes are irradiated under a Co^{60} source. The dose rate can be empirically determined. Rates of 0.01×10^5 to 1×10^6 rads (r)/h are typical and the most effective rate was 0.1×10^6 r/h. A total dose of $0.5–10\times10^6$ rads was typical and the most effective-dose was $2.6–2.9\times10^6$ rads.

Functional groups are introduced by selection of the monomers, such as styrene, choloromethylstyrene, methylacrylate, 2-hydroxymethylacrylate and/or other vinyl monomers containing one or more functional groups. For example (see, e.g., FIG. 33) aminomethyl functional groups been introduced by first radiation grafting polystyrene onto the surface of tubes of the above-noted polymers tubes followed by functionalization using N-(hydroxymethyl) phthal-imide with trifluoromethanesulfonic acid as a catalyst. The polystyrene grafted polymer tube is thoroughly washed before use to remove residual monomer, non-attached polystyrene and additives remaining from the radiation grafting. The amidoalkylation proceeds smoothly at room temperature in 50% (v/v) trifluoroacetic acid-dichloromethane solvent for 24 hours. Loading can be controlled by changing the concentrations of reagent, catalyst and/or reaction time. Hydrazinolysis in refluxing ethanol gives the aminomethyl polystyrene grafted polymer tube. Adjustable loading range is on the order of 0.5–100 μmol per tube, depending the size of the tube and the polymer.

A carboxylic acid group was introduced by using acrylate acid or functionalization of polystyrene. The polystyrene grafted tube was functionalized using n-butylithium and N,N N',N'-tetramethylethylendiamine in hexane at 60° C., after which the polymer tube was bubbled with CO_2. The carboxylic acid loading was about 1–20 μmol per tube.

Also larger matrix particles, which advantageously provide ease of handling, may be used and may be in contact with or proximity to more than one memory (i.e., one particle may have a plurality of memories in proximity or linked to it; each memory may programmed with different data regarding the matrix particle, linked molecules, synthesis or assay protocol, etc.). Thus, so-called macro-beads (Rapp Polymere, Tubingen, Germany), which have a diameter of 2 mm when swollen, or other matrices of such size, are also contemplated for use herein. Particles of such size can be readily manipulated and the memory can be readily

impregnated in or on the bead. These beads (available from Rapp) are also advantageous because of their uniformity in size, which is useful when automating the processes for electronically tagging and assaying the beads.

The matrices may also include an inert strip, such as a polytetrafluoro-ethylene [TEFLON®] strip or other material to which the molecules or biological particles of interest do not adhere, to aid in handling the matrix, such as embodiments in which a matrix with memory and linked molecules or biological particle are introduced into an agar-containing plate for immunoassays or for antibiotic screening.

Selection of the matrices will be governed, at least in part, by their physical and chemical properties, such as solubility, functional groups, mechanical stability, surface area swelling propensity, hydrophobic or hydrophilic properties and intended use.

The data storage device with programmable memory may be coated with a material, such as a glass or a plastic, that can be further derivatized and used as the support or it may be encased, partially or completely, in the matrix material, such as during or prior to polymerization of the material. Such coating may be performed manually or may be automated. The coating can be effected manually or using instruments designed for coating such devices. Instruments for this purpose are available Isee, e.g., the Series C3000 systems for dipping available from Specialty Coating Systems, Inc., Indianapolis, Ind.; and the Series CM 2000 systems for spray coating available from Integrated Technologies, Inc. Acushnet, Mass.].

The data storage device with memory may be physically inserted into the matrix material or particle. It also can be manufactured with a coating that is suitable for use as a matrix or that includes regions in the coating that are suitable for use as a matrix. If the matrix material is a porous membrane, it may be placed inside the membrane. It is understood that when the memory device is encased in the matrix or coated with protective material, such matrix or material must be transparent to the signal used to program the memory for writing or reading data. More than one matrix particle may be linked to each data storage device.

In some instances, the data storage device with memory is coated with a polymer, which is then treated to contain an appropriate reactive moiety or in some cases the device may be obtained commercially already containing the reactive moiety, and may thereby serve as the matrix support upon which molecules or biological particles are linked. Materials containing reactive surface moieties such as amino silane linkages, hydroxyl linkages or carboxysilane linkages may be produced by well established surface chemistry techniques involving silanization reactions, or the like. Examples of these materials are those having surface silicon oxide moieties, covalently linked to gamma-aminopropylsilane, and other organic moieties; N-[3-(triethyoxysilyl)-propyl]phthelamic acid; and bis-(2-hydroxyethyl)aminopropyltriethoxysilane. Exemplary of readily available materials containing amino group reactive functionalities, include, but are not limited to, para-aminophenyltriethyoxysilane. Also derivatized polystyrenes and other such polymers are well known and readily available to those of skill in this art (e.g., the TENTAGEL® Resins are available with a multitude of functional groups, and are sold by Rapp Polymere, Tubingen, Germany; see, U.S. Pat. No. 4,908,405 and U.S. Pat. No. 5,292,814; see, also Butz et al. (1994) *Peptide Res.* 7:20–23; Kleine et al. (1994) *Immunobiol.* 190:53–66].

The data storage device with memory, however, generally should not or cannot be exposed to the reaction solution, and, thus, must be coated with at least a thin layer of a glass or ceramic or other protective coating that does not interfere with the operation of the device. These operations include electrical conduction across the device and transmission of remotely transmitted electromagnetic radiation by which data are written and read. It is such coating that may also serve as a matrix upon which the molecules or biological particles may be linked.

The data storage devices with memory may be coated either directly or following coating with a ceramic, glass or other material, may then be coated with agarose, which is heated, the devices are dipped into the agarose, and then cooled to about room temperature. The resulting glass, silica, agarose or other coated memory device, may be used as the matrix supports for chemical syntheses and reactions.

Conventional integrated circuit manufacturing and packaging methods include methods and means for encapsulating integrated circuits to protect the devices from the environment and to facilitate connection to external devices. Also, there are numerous descriptions for the preparation of semiconductor devices and wires, particularly for use as sensors [see, e.g., U.S. Pat. No. 4,933,285; see, also Cass, Ed. (1990) *Biosensors A Practical Approach*, IRL Press at Oxford University Press, Oxford; chemosensors are sensors that can include a biological or chemical detection system, generally biologically active substances, such as enzymes, antibodies, lectins and hormone receptors, which are immobilized on the surface of the sensor electrode or in a thin layer on the sensor electrode; biosensors are sensors that detect biological species and for purposes herein can be implanted in an animal], which measure electrochemical solution parameters, such as pH. Despite differences in the components of biosensors and recording devices used herein, certain of the methods for coating electrodes and wires in the biosensor art may be adapted for use herein [see, e.g., U.S. Pat. Nos. 5,342,772, 5,389,534, 5,384,028, 5,296,122, 5,334,880, 5,311,039, 4,777,019, 5,143,854, 5,200,051, 5,212,050, 5,310,686, 5324,591; see, also Usmani et al., ed. (1994) *Diagnostic Biosensor Polymers*, ACS Symposium Series No. 556].

It is, however, emphasized that the combinations herein of matrix with memory are not sensors, which measure external parameters and can include electrodes that must be in contact with the solution such that molecules in solution directly contact the electrode, and which measure solution parameters. Data regarding the combination, particularly the linked or associated biological particle or matrix is written into the memory, and thus records information about itself. Sensors monitor what is going outside of the device. The combinations herein of matrices with memories can be enhanced by addition of sensor elements for the measurement of external conditions, information about the external conditions can be recorded into the combination's memory.

The combinations herein are matrix materials with recording devices that contain data storage units that include remotely programmable memories; the recording devices used in solution must be coated with a material that prevents contact between the recording device and the medium, such as the solution or air or gas [e.g., nitrogen or oxygen or CO_2]. The information is introduced into the memory by addressing the memory to record information regarding molecules or biological particles linked thereto. Except in the reaction detecting [verifying] embodiment, in which the memory can be encoded upon reaction of a linked molecule or biological particle, solution parameters are not recorded in the memory.

In certain embodiments herein, the matrices with memories herein, however may be combined with devices or

components or biosensors or other such sensor devices and used in connection therewith to monitor solution or external parameters. For example, the combination may be electronically or otherwise linked to a biosensor and information obtained by the biosensor can be encoded in memory, or the combination can transmit information to the biosensor or, when used internally in an animal, to monitor the location of a biosensor or to transmit information from the biosensor. For example, transponder memory devices exemplified herein, include circuitry for measuring and recording solution temperature. These transponders can be modified to read and record pH, instead of or in addition to temperature. Thus, during synthesis or other processing steps of linked or proximate molecules or biological particles, RF or other EM radiation will be used to encode information in the memory and at the same time pH and/or temperature in the external solution can be measured and recorded in the memory.

1. Natural Matrix Support Materials

Naturally-occurring supports include, but are not limited to agarose, other polysaccharides, collagen, celluloses and derivatives thereof, glass, silica, and alumina. Methods for isolation, modification and treatment to render them suitable for use as supports is well known to those of skill in this art [see, e.g., Hermanson et al. (1992) *Immobilized Affinity Ligand Techniques*, Academic Press, Inc., San Diego]. Gels, such as agarose, can be readily adapted for use herein. Natural polymers such as polypeptides, proteins and carbohydrates; metalloids, such as silicon and germanium, that have semiconductive properties, as long as they do not interfere with operation of the data storage device may also be adapted for use herein. Also, metals such as platinum, gold, nickel, copper, zinc, tin, palladium, silver, again as long as the combination of the data storage device with memory, matrix support with molecule or biological particle does not interfere with operation of the device with memory, may be adapted for use herein. Other matrices of interest include oxides of the metal and metalloids, such as, but not limited to, Pt—PtO, Si—SiO, Au—AuO, TiO2 and Cu—CuO. Also compound semiconductors, such as lithium niobate, gallium arsenide and indium-phosphide, and nickel-coated mica surfaces, as used in preparation of molecules for observation in an atomic force microscope [see, e.g., III et al. (1993) *Biophys J.* 64:919] may be used as matrices. Methods for preparation of such matrix materials are well known.

For example, U.S. Pat. No. 4,175,183 describes a water insoluble hydroxyalkylated cross-linked regenerated cellulose and a method for its preparation. A method of preparing the product using near stoichiometric proportions of reagents is described. Use of the product directly in gel chromatography and as an intermediate in the preparation of ion exchangers is also described.

2. Synthetic Matrices

There are innumerable synthetic matrices and methods for their preparation known to those of skill in this art. Synthetic matrices are typically produced by polymerization of functional matrices, or copolymerization from two or more monomers of from a synthetic monomer and naturally occurring matrix monomer or polymer, such as agarose. Before such polymers solidify, they are contacted with the data storage device with memory, which can be cast into the material or dipped into the material. Alternatively, after preparation of particles or larger synthetic matrices, the recording device containing the data storage unit(s) can be manually inserted into the matrix material. Again, such devices can be pre-coated with glass, ceramic, silica or other suitable material.

Synthetic matrices include, but are not limited to: acrylamides, dextran-derivatives and dextran co-polymers, agarose-polyacrylamide blends, other polymers and co-polymers with various functional groups, methacrylate derivatives and co-polymers, polystyrene and polystyrene copolymers [see, e.g., Merrifield (1964) Biochemistry 3:1385–1390; Berg et al. (1990) in *Innovation Perspect. Solid Phase Synth. Collect. Pap.*, Int. Symp., 1st, Epton, Roger (Ed), pp. 453–459; Berg et al. (1989) in *Pept., Proc. Eur. Pept. Symp.*, 20th, Jung, G. et al. (Eds), pp. 196–198; Berg et al. (1989) *J. Am. Chem. Soc.* 111:8024–8026; Kent et al. (1979) *Isr. J. Chem.* 17:243–247; Kent et al. (1978) *J. Org. Chem.* 43:2845–2852; Mitchell et al. (1976) *Tetrahedron Lett.* 42:3795–3798; U.S. Pat. No. 4,507,230; U.S. Pat. No. 4,006,117; and U.S. Pat. No. 5,389,449]. Methods for preparation of such matrices are well-known to those of skill in this art.

Synthetic matrices include those made from polymers and co-polymers such as polyvinylalcohols, acrylates and acrylic acids such as poly-ethylene-co-acrylic acid, polyethylene-co-methacrylic acid, polyethylene-co-ethylacrylate, polyethylene-co-methyl acrylate, polypropylene-co-acrylic acid, polypropylene-co-methyl-acrylic acid, polypropylene-co-ethylacrylate, polypropylene-co-methyl acrylate, polyethylene-co-vinyl acetate, poly-propylene-co-vinyl acetate, and those containing acid anhydride groups such as polyethylene-co-maleic anhydride, polypropylene-co-maleic anhydride and the like. Liposomes have also been used as solid supports for affinity purifications [Powell et al. (1989) *Biotechnol. Bioeng.* 33:173].

For example, U.S. Pat. No. 5,403,750, describes the preparation of polyurethane-based polymers. U.S. Pat. No. 4,241,537 describes a plant growth medium containing a hydrophilic polyurethane gel composition prepared from chain-extended polyols; random copolymerization is preferred with up to 50% propylene oxide units so that the prepolymer will be a liquid at room temperature. U.S. Pat. No. 3,939,123 describes lightly crosslinked polyurethane polymers of isocyanate terminated prepolymers containing poly(ethyleneoxy) glycols with up to 35% of a poly (propyleneoxy) glycol or a poly(butyleneoxy) glycol. In producing these polymers, an organic polyamine is used as a crosslinking agent. Other matrices and preparation thereof are described in U.S. Pat. Nos. 4,177,038, 4,175,183, 4,439, 585, 4,485,227, 4,569,981, 5,092,992, 5,334,640, 5,328,603.

U.S. Pat. No. 4,162,355 describes a polymer suitable for use in affinity chromatography, which is a polymer of an aminimide and a vinyl compound having at least one pendant halo-methyl group. An amine ligand, which affords sites for binding in affinity chromatography is coupled to the polymer by reaction with a portion of the pendant halo-methyl groups and the remainder of the pendant halo-methyl groups are reacted with an amine containing a pendant hydrophilic group. A method of coating a substrate with this polymer is also described. An exemplary aminimide is 1,1-dimethyl-1-(2-hydroxyoctyl)amine methacrylimide and vinyl compound is a chloromethyl styrene.

U.S. Pat. No. 4,171,412 describes specific matrices based on hydrophilic polymeric gels, preferably of a macroporous character, which carry covalently bonded D-amino acids or peptides that contain D-amino acid units. The basic support is prepared by copolymerization of hydroxyalkyl esters or hydroxyalkylamides of acrylic and methacrylic acid with

crosslinking acrylate or methacrylate comonomers are modified by the reaction with diamines, aminoacids or dicarboxylic acids and the resulting carboxyterminal or aminoterminal groups are condensed with D-analogs of aminoacids or peptides. The peptide containing D-aminoacids also can be synthesized stepwise on the surface of the carrier.

U.S. Pat. No. 4,178,439 describes a cationic ion exchanger and a method for preparation thereof. U.S. Pat. No. 4,180,524 describes chemical syntheses on a silica support.

Immobilized Artificial Membranes [IAMs; see, e.g., U.S. Pat. Nos. 4,931,498 and 4,927,879] may also be used. IAMs mimic cell membrane environments and may be used to bind molecules that preferentially associate with cell membranes [see, e.g., Pidgeon et al. (1990) *Enzyme Microb. Technol.* 12:149].

3. Immobilization and Activation

Numerous methods have been developed for the immobilization of proteins and other biomolecules onto solid or liquid supports [see, e.g., Mosbach (1976) *Methods in Enzymology* 44; Weetall (1975) *Immobilized Enzymes, Antigens, Antibodies, and Peptides*; and Kennedy et al. (1983) *Solid Phase Biochemistry, Analytical and Synthetic Aspects*, Scouten, ed., pp. 253–391; see, generally, *Affinity Techniques, Enzyme Purification: Part B. Methods in Enzymology*, Vol. 34, ed. W. B. Jakoby, M. Wilchek, Acad. Press, N.Y. (1974); *Immobilized Biochemicals and Affinity Chromatography, Advances in Experimental Medicine and Biology*, vol. 42, ed. R. Dunlap, Plenum Press, N.Y. (1974)].

Among the most commonly used methods are absorption and adsorption or covalent binding to the support, either directly or via a linker, such as the numerous disulfide linkages, thioether bonds, hindered disulfide bonds, and covalent bonds between free reactive groups, such as amine and thiol groups, known to those of skill in art [see, e.g., the PIERCE CATALOG, ImmunoTechnology Catalog & Handbook, 1992–1993, which describes the preparation of and use of such reagents and provides a commercial source for such reagents; and Wong (1993) *Chemistry of Protein Conjugation and Cross Linking*, CRC Press; see, also DeWitt et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Zuckermann et al. (1992) *J. Am. Chem. Soc.* 114:10646; Kurth et al. (1994) *J. Am. Chem. Soc.* 116:2661; Ellman et al. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91:4708; Sucholeiki (1994) *Tetrahedron Lttrs.* 35:7307; and Su-Sun Wang (1976) *J. Org. Chem.* 41:3258; Padwa et al. (1971) *J. Org. Chem.* 41:3550 and Vedejs et al. (1984) *J. Org. Chem.* 49:575, which describe photosensitive linkers].

To effect immobilization, a solution of the protein or other biomolecule is contacted with a support material such as alumina, carbon, an ion-exchange resin, cellulose, glass or a ceramic. Fluorocarbon polymers have been used as supports to which biomolecules have been attached by adsorption [see, U.S. Pat. No. 3,843,443; Published International PCT Application WO/86 03840].

A large variety of methods are known for attaching biological molecules, including proteins and nucleic acids, molecules to solid supports [see. e.g., U.S. Pat. No. 5,451, 683]. For example, U.S. Pat. No. 4,681,870 describes a method for introducing free amino or carboxyl groups onto a silica matrix. These groups may subsequently be covalently linked to other groups, such as a protein or other anti-ligand, in the presence of a carbodiimide. Alternatively, a silica matrix may be activated by treatment with a cyanogen halide under alkaline conditions. The anti-ligand is covalently attached to the surface upon addition to the activated surface. Another method involves modification of a polymer surface through the successive application of multiple layers of biotin, avidin and extenders [see, e.g., U.S. Pat. No. 4,282,287]; other methods involve photoactivation in which a polypeptide chain is attached to a solid substrate by incorporating a light-sensitive unnatural amino acid group into the polypeptide chain and exposing the product to low-energy ultraviolet light [see, e.g., U.S. Pat. No. 4,762,8811]. Oligonucleotides have also been attached using a photochemically active reagents, such as a psoralen compound, and a coupling agent, which attaches the photoreagent to the substrate [see, e.g., U.S. Pat. No. 4,542,102 and U.S. Pat. No. 4,562,157]. Photoactivation of the photoreagent binds a nucleic acid molecule to the substrate to give a surface-bound probe.

Covalent binding of the protein or other biomolecule or organic molecule or biological particle to chemically activated solid matrix supports such as glass, synthetic polymers, and cross-linked polysaccharides is a more frequently used immobilization technique. The molecule or biological particle may be directly linked to the matrix support or linked via linker, such as a metal [see, e.g., U.S. Pat. No. 4,179,402; and Smith et al. (1992) *Methods: A Companion to Methods in Enz.* 4:73–78]. An example of this method is the cyanogen bromide activation of polysaccharide supports, such as agarose. The use of perfluorocarbon polymer-based supports for enzyme immobilization and affinity chromatography is described in U.S. Pat. No. 4,885, 250]. In this method the biomolecule is first modified by reaction with a perfluoroalkylating agent such as perfluorooctylpropylisocyanate described in U.S. Pat. No. 4,954, 444. Then, the modified protein is adsorbed onto the fluorocarbon support to effect immobilization.

The activation and use of matrices are well known and may be effected by any such known methods [see, e.g., Hermanson et al. (1992) *Immobilized Affinity Ligand Techniques*, Academic Press, Inc., San Diego]. For example, the coupling of the amino acids may be accomplished by techniques familiar to those in the art and provided, for example, in Stewart and Young, 1984, *Solid Phase Synthesis*, Second Edition, Pierce Chemical Co., Rockford.

Molecules may also be attached to matrices through kinetically inert metal ion linkages, such as Co(III), using, for example, native metal binding sites on the molecules, such as IgG binding sequences, or genetically modified proteins that bind metal ions [see, e.g., Smith et al. (1992) *Methods: A Companion to Methods in Enzymology* 4, 73 (1992); III et al. (1993) *Biophys J.* 64:919; Loetscher et al. (1992) *J. Chromatography* 595:113–199; U.S. Pat. No. 5,443,816; Hale (1995) *Analytical Biochem.* 231:46–49].

Other suitable methods for linking molecules and biological particles to solid supports are well known to those of skill in this art [see, e.g., U.S. Pat. No. 5,416,193]. These linkers include linkers that are suitable for chemically linking molecules, such as proteins and nucleic acid, to supports include, but are not limited to, disulfide bonds, thioether bonds, hindered disulfide bonds, and covalent bonds between free reactive groups, such as amine and thiol groups. These bonds can be produced using heterobifunctional reagents to produce reactive thiol groups on one or both of the moieties and then reacting the thiol groups on one moiety with reactive thiol groups or amine groups to which reactive maleimido groups or thiol groups can be attached on the other. Other linkers include, acid cleavable linkers, such as bismaleimideothoxy propane, acid labile-

transferrin conjugates and adipic acid diihydrazide, that would be cleaved in more acidic intracellular compartments; cross linkers that are cleaved upon exposure to UV or visible light and linkers, such as the various domains, such as C_H1, C_H2, and C_H3, from the constant region of human IgG_1 (see, Batra et al. (1993) *Molecular Immunol.* 30:379–386).

Presently preferred linkages are direct linkages effected by adsorbing the molecule or biological particle to the surface of the matrix. Other preferred linkages are photocleavable linkages that can be activated by exposure to light [see, e.g., Baldwin et al. (1995) *J. Am. Chem. Soc.* 117:5588; Goldmacher et al. (1992) *Bioconj. Chem.* 3:104–107]. The photocleavable linker is selected such that the cleaving wavelength that does not damage linked moieties. Photocleavable linkers are linkers that are cleaved upon exposure to light [see, e.g., Hazum et al. (1981) in *Pept., Proc. Eur. Pept. Symp.*, 16*th*, Brunfeldt, K (Ed, pp. 105–110, which describes the use of a nitrobenzyl group as a photocleavable protective group for cysteine; Yen et al. (1989) *Makromol. Chem* 190:69–82, which describes water soluble photocleavable copolymers, including hydroxypropylmethacrylamide copolymer, glycine copolymer, fluorescein copolymer and methylrhodamine copolymer; Goldmacher et al. (1992) *Bioconj. Chem.* 3:104–107, which describes a cross-linker and reagent that undergoes photolytic degradation upon exposure to near UV light (350 nm); and Senter et al. (1985) *Photochem. Photobiol* 42:231–237, which describes nitrobenzyloxycarbonyl chloride cross linking reagents that produce photocleavable linkages]. Other linkers include fluoride labile linkers [see, e.g., Rodolph et al. (1995) *J. Am. Chem. Soc.* 117:5712], and acid labile linkers [see, e.g., Kick et al. (1995) *J. Med. Chem.* 38:1427]. The selected linker will depend upon the particular application and, if needed, may be empirically selected.

B. Optically encoded memory devices

The matrices or strips attached thereto may be encoded with a pre-programmed identifying bar code, such as an optical bar code that will be encoded on the matrix and read by laser. Such pre-coded devices may be used in embodiments in which parameters, such as location in an automated synthesizer, are monitored. The identity of a product or reactant determined by its location or path, which is monitored by reading the chip or memory in each device and storing such information in a remote computer.

Thus, it is contemplated herein, that the memory is not proximate to the matrix, but is separate, such a memory in a remote computer or other recording device. In these embodiments, the matrices are marked with a unique code or mark of any sort. The identity of each mark is saved in the remote memory, and then, each time something is done to a molecule or biological particle linked to each matrix, the information regarding such event is recorded and associated with the coded identity. After completion of, for example, a synthetic protocol, each matrix is examined or read to identify the code. Retrieving information that from the remote memory that is stored with the identifying code will permit identification or retrieval of any other saved information regarding the matrix.

For example, simple codes, including bar codes, alphanumeric characters or other visually or identifiable codes or marks on matrices are also contemplated for use herein. When bar codes or other precoded devices are used, the information can be written to an associated but remote memory, such as a computer or even a piece of paper. The computer stores the bar code that a identifies a matrix particle or other code and information relating to the molecule or biological particle linked to the matrix or other relevant information regarding the linked materials or synthesis or assay. Instead of writing to an on-board memory, information is encoded in a remote memory that stores information regarding the precoded identity of each matrix with bar code and linked molecules or biological particles. Thus, the precoded information is associated with, for example, the identity of the linked molecule or a component thereof, or a position (such as X-Y coordinates in a grid). This information is transmitted to a memory for later retrieval. Each treatment or synthetic step that is performed on the linked molecule or biological particle is transmitted to the remote memory and associated with the precoded ID.

For example, an amino acid is linked to a matrix particle that is encoded with or marked with a bar code or even a letter such as "A" or other coded mark. The identity the amino acid linked to the matrix particle "A" is recorded into a memory. This particle is mixed with other particles, each with a unique identifier or mark, and this mixture is then treated to a synthetic step. Each particle is individually scanned or viewed to see what mark is on each particle and the remote memory is written to describe the synthetic step, which is then associated with each unique identifier in the memory, such as the computer or piece of paper. Thus, in the remote memory the original amino acid linked to particle A is stored. After the synthetic step, the identify of the next amino acid is stored in the memory associated with "A" as is the identity of the next amino acid added. At the end of the synthesis, the history of each particle can be read by scanning the particle or visually looking at the particle and noting its bar code or mark, such as A. The remote memory is then queried to determine what amino acids are linked to the particle identified as "A" [see, e.g., FIG. **20**].

For example, many combinatorial libraries contain a relatively small number of discrete compounds [10^{2-104}] in a conveniently manipulable quantity, rather than millions of members in minute quantities. These small libraries are ideal for use with the methods and matrices with memories herein. They may also be used in methods in which the memory is not in proximity to the matrix, but is a remote memory, such as a computer or a table of information stored even on paper. The system depicted in FIG. **20** is ideal for use in these methods. Polypropylene or other inert polymer, including fluoropolymers or scintillating polymers are molded into a convenient geometry and size, such an approximately 5 mmx5 mmx5 mm cube [or smaller or larger] with a unique identifying code imprinted, preferably permanently, on one side of each cube. If, for example, a three element code is used, based on all digits (0to 9) and all letters of the alphabet, a collection of 46,666 unique three element codes are available for imprinting on the cubes.

The cubes are surface grafted with a selected monomer [or mixture of monomer], such as styrene. Functionalization of the resulting polymer provides a relatively large surface area for chemical syntheses and subsequent assaying [on a single platform]. For example, a 5×5×5 mm^3 cube has a surface area of 150 mm^2, which is equivalent to about 2–5 μmol achievable loading, which is about 1–2.5 mg of compounds with a molecular weight of about 500. A computer program, described below [see, e.g., Examples], or protocol can direct split and pool during synthesis and the information regarding each building block of the linked molecules on each cube conveniently recorded in the memory [i.e., computer] at each step in the synthesis.

Since the cubes [herein called MACROCUBES™ or MACROBEADS™] are relatively large, they can be read by the eye or any suitable device during synthesis and the

associated data can be manually entered into a computer or even written down. The cubes can include scintillant or fluorophore or label and used in any of the assay formats described herein or otherwise known to those of skill in the art.

For example, with reference to FIG. **20**, polypropylene, polyethylene or fluophore raw material [any such material described herein, particularly the Moplen resin e.g., V29G PP resin from Montell, Newark Del., a distributor for Himont, Italy] **1** is molded, preferably into a cube, preferably about 5x5x5 mm^3 and engraved, using any suitable imprinting method, with a code, preferably a three element alphanumeric code, on one side. The cube can be weighted or molded so that it all cubes will orient in the same direction. The engraved cubes **2** are then surface-grafted **3** and functionalized using methods described herein or known to those of skill in this art, to produce cubes [MACROBEADS™ or MACROCUBES™] or devices any selected geometry **4**.

1. Encoded Memory Devices With Two-dimensional Bar Codes

a. Matrices With Optical Memories

In another exemplary embodiment, illustrated in FIG. **22**, the optical memory device ["OMD"] **100** is a preferably a rectangular parallelepiped that provides a broad face upon which encoded information can be inscribed. Any geometry that is suitable for a particular application and that provides at least one surface for encoding information. The OMDs may also be containers used for chemical synthesis, such as microtiter plates, tubes, tubes adapted for use with microtiter-type plates. The two-dimensional bar code described herein is ideally suited for incorporation onto the outside surface of each well of a microtiter plate or on the outside of a small test tube or other such tube, particularly, tubes intended for use with a microplate frame, such as those available from NUNC and COSTAR. This two-dimensional bar code as wells as the method for reading and writing may also be used to track and identify other laboratory equipment, such as chromatography tubes, test tubes, beakers, flasks and other such items.

The OMDs may also be fabricated as tubes, such as the MICROTUBES™ provided herein. When used with such tubular devices, they will be engraved on the outer surface, preferably the top or bottom of the device.

The material of which the OMDs are fabricated will depend upon the monitored processes. The materials that may be used include, but are not limited to, black, white or colored glass, TEFLON®, polyethylene, high density polyethylene, polypropylene, polystyrene, polyester, ceramic, such as alumina or zirconia, metal, or any composite of the above materials or any material that is physically or chemically structured to produce optical contrast as the result of exposure to the write process, which is described below. For use in the methods herein, these materials may be suitable or at least one surface there may have been treated to render them suitable for retaining molecules and biological particles for use as matrices as described herein.

For the first exemplary embodiment of OMD **100** shown in FIG. **22**, if the OMD is formed from a ceramic material, it may have exemplary dimensions of 280 mil (L)x140 mil (W)x50 mil (T) [7 mmx3.5 mmx1.3 mm]. The dimensions of the face can be varied as needed to provide the appropriate size for recording data, providing sufficient chemical binding surface area, and to facilitate handling. The presently preferred minimum size for use with commercial feeding systems is on the order of 0.5 mmx0.5 mmx0.5 mm.

If the OMD **100** is formed from polypropylene, it may have exemplary dimensions of 280 mil (L)x140 mil (W)x 100 mil (T) [7 mmx3.5 mmx2.6 mm], although smaller dimensions are contemplated. Since OMDs made from polypropylene may be read by transmission of light through the device, the thickness must be sufficiently thin to permit transmission of light through the OMD, except where there are darkened areas of a bar code symbol. Where reflected light is to be used, as with the ceramic OMDs, thickness need not be so limited.

For OMDs used for chemical binding or other processes for which surfaces must be specially prepared in order to assure adsorption or absorption or any means of binding of molecules or biological particles, it may be desirable to separate the binding surfaces from the data storage surface **101**. In this case, one or more of sides **104** and **105**, bottom **107**, top **108**, and back **110** may be treated to enhance binding using radiation, mechanical or chemical abrasion, or other processes as appropriate. By segregation of the binding and information surfaces, possible activation or modification of certain bound compounds by the high intensity light source used in the write process is avoided. In addition, degradation of the bar code contrast may be less on a surface that is not derivatized for binding.

If needed, segregation of the binding and information surfaces can be achieved by coating portions of the OMD with films formed from a dielectric material such as polyethylene, MYLAR, TEFLON®, KAPTON, polycarbonate, or, preferably, the para-xylylene polymers sold under the trade name Parylene [see, e.g., U.S. Pat. Nos. 3,288,728, 3,342,754 and 3,429,739], or any other such materials that are commonly used in the electronics industry to passivate electronic components and circuit boards, and as a coating for medical devices, especially implants, catheters, probes and needles. [Parylene is the trade name for members of a series of polymers which are commercially available from Specialty Coating Systems, Inc., of Indianapolis, Ind. and originally from Union Carbide Corporation, Greenville, S.C., see, U.S. Pat. Nos. 3,288,728, 3,342,754 and Gorham 3,429,739; see, also brochures distributed by the manufacturer, entitled "Parylene Conformal Coatings Specifications and Properties" (©1984, Specialty Coating Systems, Inc.), and "Parylene, A Biostable Coating for Medical Applications" (©1984, Specialty Coating Systems, Inc.]. These polymers provide a conformal biostable coating which electrically and chemically isolates the protected surface from its environment.

The Parylene or other such polymeric coating can be treated to form a chemically functional substrate by methods such as beta or gamma radiation, and mechanical or chemical roughening. Alternatively, polystyrene microspheres can be bonded [glued or welded] to selected surface(s) of the OMD, either on the Parylene or similar coating, or directly to the ceramic or polypropylene.

The encoded information may be stored in any optically writable and readable format. As shown printed on data storage surface **101**, symbologies **106** are two-dimensional bar codes, which can be stacked rows of one-dimensional bar codes, checkerboards, or dot matrices. Other symbologies that can be used include one-dimensional bar codes, target codes, alphanumeric characters or other optically readable characters which are well known in the art. [See, e.g., Wang, et al. (1990) *A High Density Two Dimensional*

Bar Code SPIE Proceedings Vol. 1384, High-Speed Inspection Architectures, Bar Coding, and Character Recognition, pp. 169–175; Martin (1991) *Unique Symbol for Marking and Tracking Very Small Semiconductor Products, SPIE Proceedings* Vol. 1598, Lasers in Microelectronic Manufacturing, pp. 206–220.]

In the exemplary embodiment, the two-dimensional bar code [e.q., symbol **106**] includes an orientation indicator in the form of solid black lines across the top **120** and down the right side **122** of the symbol. Upon acquisition of the image of the symbol by the image sensing means, the image processor will utilize the orientation indicator to provide information about the rotation of the OMD relative to the sensor, and can compensate in its software by rotating the image to the appropriate orientation for decoding the image. Other types of orientation indicators as are known in the art, such as those described in the above-identified references relating to bar codes, may also be used such that physical precise orientation of the OMD within the read area is not critical. For reflection-type readers, it is only necessary for the OMD to be right side up and the symbol is fully within the field of view of the detector, so that the symbol **106** is exposed to the image sensor. Even where reading is accomplished by transmission of light through the OMD, as in certain polypropylene embodiments, an orientation indicator in the symbol in combination with a distinctive physical or optical feature, such as described below, can provide information sufficient to determine whether the OMD is face up or face down so that appropriate compensation, such as reversal of the image, can be performed by the software in order to enable decoding.

An alternative means for recording and reading information involves the formation of a magnetic film on at least a portion of the surface of the OMD. Creation of thin magnetic films by sputtering, electroplating, or other deposition techniques are well known in magnetic recording technology. [See, e.g., Chapter 11, "Tape and Disk Materials" from *The Complete Handbook of Magnetic Recording*, 3rd Edition, by Finn Jorgenson, Tab Books, 1988.] Recording and reading of data on the magnetic film can utilize conventional magnetic recording techniques.

The OMD **200** of FIG. **23** is a variation on the embodiment of FIG. **22** that provides a information recording section that is formed from a separate material from that of the binding surface(s) [i.e., the chemistry surface(s) or the surface(s) to which molecules or biological particles are linked]. In this embodiment, the OMD contains two sections that are linked together. Here, OMD **200** is formed from the assembly of information unit **202** and binding unit **204**, with unit **202** fitting within a cavity or well **206** formed in unit **204**. This embodiment provides the advantage of selecting the optimal material for each of the binding and recording processes, and also permits the information unit **202** to be assembled with the binding unit **204** after the binding unit has been treated to enhance adhesion. For example, binding unit **204** can be formed from a polymer, e.g., polypropylene, functionalized by radiation and/or chemical processes, or can be modified by bonding polystyrene microspheres to its surface(s). Information unit **202** can be formed from plastic, ceramic or glass, and mounted within well **206** by adhesive or other bonding process, or may simply be press fit into the well. Since pre-treatment of the binding unit to enhance binding could possibly discolor the information unit, or otherwise make it less readable by modifying the surface, e.g., pitting or etching, separate formation could be advantageous. The outer dimensions of unit **202** are preferably selected to closely fit the inside dimensions of well **206** to prevent the intrusion of chemicals, or even air, into spaces between the units. In the illustrated example, unit **204** has outer dimensions of 280 mil (L)×140 mil (W)×100 mil (T) [7 mm×3.5 mm×2.6 mm] and unit **202** has maximum dimensions of 210 mil (L)×115 mil (W)×50 mil (T) [5.3 mm×2.9 mm×1.3 mm]. Since, as shown, the sides of unit **202** are beveled to form a trapezoidal cross-section to conform to a corresponding shape of the well **206**, and also to assist in forming a tight seal between the two units, the actual exposed face of the information unit is on the order of 105 mil×200 mil [2.7 mm×5 mm]. When the two units are assembled, the combined face surface of the information and binding units are preferably flush. As can be seen, the encoded information, shown as a two-dimensional bar code symbol **208**, is inscribed on information unit **202** only. With regard to the magnetic recording alternative method, the use of a separate information unit is ideal since it would generally be preferred to avoid exposure of magnetic recording media to the radiation or corrosive chemicals used for enhancement of the binding process.

Variations on the two-part OMD of FIG. **23** are illustrated in FIGS. **25–27**. In FIG. **25**, OMD **400** is illustrated where insert unit **402** is the binding unit formed, for example, from polymer functionalized by radiation and/or derivatized by suitable chemical processes or grafted to render the surface suitable for binding biological particles and molecules. Base unit **404**, which may be formed from plastic, polymer, ceramic or glass, has a well **406** corresponding to the exterior shape of the binding unit **402**, so that they will interfit closely. The encoded information, shown, again, as a two-dimensional bar code symbol **412**, is inscribed on the back **408** of base unit **404**, opposite the face **410** at which binding unit **402** is exposed.

In FIG. **26** [an embodiment of a microvessel], insert unit **502** has a cavity **508** covered by mesh **510** [porous material] for retaining particles but permitting chemical materials and biological particles to pass through, to form OMD **500**. The chemicals pass through mesh **510** to be within cavity **508**, or some material contained therein, such as microspheres, or are retained on the strands of mesh **510**. As in the embodiment of FIG. **25**, base unit **504**, which is encoded with the symbology, receives the binding [chemistry] unit so that it is exposed on one face **512**, with the encoded information **514** located on the opposite face **516**.

In the embodiment of FIG. **27**, insert unit **602** is formed from polypropylene or ceramic or other suitable material and provides the information storage face **608** for writing symbology, preferably a bar code symbol **610** on OMD **600**. Base unit **604** provides the means for binding of chemical materials, which contains cavity **612** which is filled with microspheres **614** and covered with polypropylene screen **616** or other suitable porous material. The base material is preferably polypropylene or other such material. In this embodiment, the information storage face **608** is on the opposite side of the OMD from the screen **616**.

In yet another embodiment, OMD **700**, which is illustrated in FIG. **28**, an orientation indicator is provided in the form of a notched or cut-corner **702**. In this embodiment, the corner cut-out **702** will provide information as to the rotation and inversion of OMD **700**, since, even if the OMD is face down, it will be apparent due to the unique outline of the face. The use of a physically detectable orientation indicator allows the handling equipment to readily detect improper positioning, for example, by placement of mechanical or optical edge detectors within the handling system. An improperly positioned OMD can be removed from the imaging position and placed back at the entry point into the

reading handler, or mechanical means, such as a retractable blade, can be provided to flip the OMD over if it is presented face down within the field of view of the reader. An alternative symbology **706** is illustrated which is, in this case, an alphanumeric code, which can be read and decoded using known optical character recognition (OCR) techniques.

Other types of orientation indicators that can be used include chamfers, holes and protrusions. Several different and distinctive shapes can be included on a single OMD to assist in orientation, positioning and separation of the OMDs. For example, a group of OMDs can have a cut corner for orientation of each OMD, with some of those OMDs having a tab extending from one of its sides, so that those with tabs can be separated from those without tabs, which facilitates division of the group for diversion to different containers.

Additional test media can be included in the OMD in the embodiment of FIG. **29**. Here, the OMD **800** has a plurality of wells or recesses **804** into which can be placed gels, beads, or the like for retaining additional chemical [molecules] or biological materials [biological particles], and/or chemical, biological or temperature sensors, or other such devices. Where such materials are placed in the wells **804**, the bar code symbol **806** can include information about the nature of these materials.

The embodiment of FIG. **30** is a variation on that of FIG. **29**. Here, the OMD **900** is partially hollow, and a plug **902** is formed in the side to permit access to the cavity **912**. The front **904** and/or back **908** walls of the OMD have a mesh insert **910** which provides limited access to the cavity **912** in the OMD. A chemically- or biologically-functional material [biolgocial particle or molecule], or microspheres, for example, can be placed within the cavity **912** through plug **902** so that it is exposed to the chemical or biological materials to which the OMD is exposed without allowing direct contact between the material in the cavity and the environment in which the OMD is placed. The mesh [porous material] **910** can be polypropylene or other such suitable polymer, and of a size that makes it semi-permeable, admitting the external solution without allowing the interior material to escape. Generally, the pore size will be within the range of 20 μm to 200 μm. The use of OMDs and protocols therefor are flexible and can employ a variety of shapes, configurations, and polymeric synthesis supports. The ceramic 2-D bar codes will have at least 8-bytes of information content. When etched on ceramics, they are inert to the vast majority of organic synthesis conditions, easy and reliable to use, inexpensive, and very amenable to mass production. The low cross-linking polystyrene surface graft (and other polymer grafts) on the stable and inert base polymer provides an ideal solid support for chemical synthesis with excellent functionalizability and chemokinetics. This technology can also be applied to the synthesis of other types of compounds, especially small organic molecules. Among the advantages of this technology over existing combinatorial techniques are: a) low manufacturing cost; b) non-invasive encoding; c) high encoding reliability and capacity; d) total chemistry flexibility; e) excellent chemokinetics; f) easy and clean washing between reactions; g) utilization of the highly efficient directed sorting strategy; h) delivery of pure, discrete compounds in multi-milligram scale; and i) very amenable to full automation. Integration of the laser optical synthesis OMD technology with automation will further enhance its applications in high through-put chemical synthesis and biological screening.

These devices have a wide variety of applications. For example, with reference to FIG. **33E**, the bar-coded OMDs devices are functionalized with amino groups to give functionalized OMDs devices **3** and used in the synthesis of oligomers. The first set of nucleosides modified with a succinic acid linker are coupled onto the matrices using DNA synthesizer with modified reaction vessels [suitable for use with the OMD-linked nascent oligonucleotides]. Five cycles of TCA de-blocking, sorting, tetrazole activation, coupling, capping, and oxidation are performed automatically on the machine [except the sorting] to yield oligonucleotides [hexamers] on the matrices **6**. Cleavage and deprotection under standard conditions give the oligonucleotide hexamer library **7**. The identity of each oligonucleotide is associated with the unique code in a remote memory, such as by manually entry before, during or after synthesis.

b. Reading and Writing to Matrices With Optical Memories

An exemplary read/write system is illustrated in FIG. **24**. The write system includes laser **320**, mirror **322**, and prism **324** mounted on drive shaft **325** connected at a first end to drive motor **326**. Drive shaft **325** is connected at its second end to geared linkage **328** which rotates drive shaft **329** and prism **330** in synchrony with prism **324**. The beam emitted by laser **320** follows optical path **310** to mirror **322**, where it is reflected toward prism **324**. Prism **324** rotates to scan the beam along the y-axis, i.e., up and down, so that the beam effectively shifts top to bottom by reflection from the prism faces in succession as it rotates. This beam is similarly scanned along the x-axis from left to right by reflection from the faces of prism **330** in succession as prism **330** rotates. Either or both prisms can be replaced with rotating or oscillating mirrors to achieve the same scanning pattern, in a manner similar to the scanning mechanisms used in conventional laser-based bar code scanners [see, e.g., U.S. Pat. No. 4,387,297 to Swartz, et al., entitled "Portable Laser Scanning System and Scanning Methods", and U. S. Pat. No. 4,409,470 to Shepard, et al., entitled "Narrow Bodied, Single- and Twin-Windowed Portable Laser Scanning Head for Reading Bar Code Symbols"]. Mirrors **332** and **334** provide means for directing the beam toward the OMD **300** at the appropriate level, and, thus, are positioned in consideration of the guide means, so that the beam impinges upon the desired recording surface.

As illustrated in FIG. **24**, OMD **300** has already been inscribed during an earlier process step, evidenced by the fact that symbol **306** is present and complete. Also as illustrated, symbol **316** is currently being written by the progression of laser spot **336** across the write surface, as scanned by prisms **324** and **330**. The contrasting dark and light areas of the symbols **306,316** are created by pulsing the laser **320** according to a signal provided by system controller **346**.

In an exemplary embodiment, laser **320** is a CO_2 laser, which emits light in the infrared at a wavelength of 10.6 μm. The writing process is accomplished by using a sufficiently high power beam to burn the surface of the OMD, formed of ceramic, white polypropylene or the like, to produce a dark carbon build-up corresponding to the dark lines of the symbology on the lighter colored background. The exemplary laser power is 25 W, with a spot size of 0.03 mm, burning a dot in the write surface of 0.13 mm, to create a two-dimensional bar code using a dot matrix pattern. Mirrors **322**, **332** and **334**, and prisms **324** and **330** must be coated with an appropriate IR-reflective film to avoid damage to the optics by the laser. In read systems which utilize transmission of light through the OMD, the carbon build-up will block the light, appearing as darkened areas to a sensor

on the opposite side of the OMD from the light source. In read systems which utilize back-reflection, the carbon build-up will absorb light, while the other areas will reflect the light, again creating a contrast between the inscribed and untouched areas of the surface.

Since the IR write beam is not visible, it may be desirable to use an optically-visible laser (not shown), for example, He—Ne or a diode laser which emits within the visible spectrum, or other focused light source, to emit a beam along optical path **310** to permit visual alignment. The optically-visible laser may also be used as a proximity detector to send a signal to the system controller to indicate the presence of the OMD in the write position to trigger the write process, either by reflection or by blocked transmission using conventional optical sensors. Alternatively, a separate optical detector system may also be used to detect and indicate the presence of an OMD in the write or read position.

For OMDs formed from glass or ceramic, a beam from a CO_2 laser can be used to etch the glass to produce contrasting lines by modifying the surface finish of the glass. For example, the glass can be frosted or otherwise roughened, which may assist in the binding of compounds, and which has a reduced reflectivity. Upon exposure to the high power write beam, the glass surface is partly flowed, i.e., partly melted, so that a smooth, highly reflective surface remains after the surface cools. Contrast between the frosted and flowed glass can be enhanced for reading by selecting a read wavelength which maximizes the differences in reflectivity between the two surface finishes.

Other types of lasers which may be used include neodymium-YAG [yttrium-aluminum-garnet], excimer, or any other laser capable of emitting a sufficiently high power beam to modify the material surface to produce an optically-readable contrast. Alternative lasers for the writing process include diode lasers, such as those made by Coherent, Inc. of Santa Barbara, Calif. Among those that are suitable are Model No. S-98-2000C-200-C, T or H, which emit light at 980 nm with a CW power of 2000 mW, Model No. B1-81-10C-19-30-A or W, which emit light at 808 nm with a CW power of 10,000 mW, or Model No. B1-81-15C-19-30-A or W, which emit light at 808 nm with a CW power of 15,000 mW.

Selection of the laser will depend on the material of which the OMDs are formed. For example, if an optically-reactive material is encased within a transparent glass or plastic shell, any laser capable of inducing the readable change in the optically-reactive material would be acceptable. Photochemically-reactive media, known to those of skill in the art [see, e.g., U.S. Pat. No. 5,136,572 to Bradley, entitled "Optical Data Storage System"] can be selectively activated and read by use of wavelength tunable diode lasers, such that a lesser power and/or visible light laser can be used with a more reactive recording media.

The range of movement of the laser spot **336** is limited, generally to the area of the write surface, so that the OMDs must be moved past a target area within which laser spot **336** is projected. Movement of the OMDs can be achieved by one or more sets of conveyor belts, chutes or guide rollers, each of which can be fed by a commercial-type centrifugal feeder, such as those available from Hoppmann Corporation of Chantilly, Va. and Kirchlintein, Germany. Feeders of this type are known in industry for mass handling of parts and products, including foods, pharmaceuticals, containers and hardware. Linear and vibratory feeders are also known and may be used for handling the OMDs. An exemplary handling system is illustrated in FIG. **32** and will be discussed in more detail below.

Included in the proximity location process to detect the presence of an OMD within the write position can be a detector **372** for locating the next available area on the write surface for writing. In the example illustrated in FIG. **24**, for a write surface having four available locations, position **#1** is already filled with symbology **306**, and position **#2** is in the process of being filled with symbology **316**. A light source **368**, such as a visible laser, emits a beam which is directed by scanning optics **370** toward the write surface of the OMD. As the write surface is scanned by the beam, the next available area detector **372** can look sequentially at each OMD as its presence is detected, first at position #1, then at subsequent positions until it finds an area on which no symbology is written, i.e., no contrasting markings are detected, or a "white" area of a pre-determined width is detected which is wider than the "quiet Zone" which is commonly included in bar codes. [See, e.g., Wang, et al., "*A High Density Two Dimensional Bar Code*," SPIE Proceedings Vol. 1384, High-Speed Inspection Architectures, Bar Coding, and Character Recognition (1990) pp. 169–175.] This location process permits multiple uses of OMDs, and takes into consideration that some OMDs may be exposed to a greater number of process steps than others before being combined into the string in which they are presently included.

The detector **372** can also be used for indication of the presence of OMDs to be read. In the case of reading, the detector **372** can also be used to identify the presence of all symbologies to be scanned for reading, which is particularly important if a laser beam or other relatively narrow beam of light is scanned over the written surface to read the symbology. Where an incoherent light source is used to simply flood the entire write surface with light, such as a lamp **340**, the ability to detect the presence of individual symbologies is not critical, since the entire write surface will be viewed and recorded at once using frame grabbing techniques.

During the read process, after the presence of an OMD is indicated, the lamp **340** is activated to illuminate the write surface. The light reflected from the surface is modulated by the symbology printed thereon due to the selective reflection and absorption of the contrasting areas. Optics **342**, which will typically be an assembly of lenses and filters, which remove stray light, focus the reflected light onto detector **344**. Selection of optics can be performed in a using methods known to those of skill in the art [see, e.g., U.S. Pat. No. 5,354,977 of Roustaei, which describes an optical bar code scanner using a CCD [charge-coupled device] detector and associated optics. In the same reference, a detailed description of CCD detectors for use in bar code scanners is provided, as well as steps for processing the signal generated by the CCD detector.

In the exemplary embodiment illustrated in FIG. **24**, the CCD detector **344** comprises an array of discrete devices, each of which is a "pixel", capable of storing charge impinging upon it representative of reflected light from the write surface, then reading out the charge as a serial analog waveform. A typical CCD array for bar code scanning has 2048 pixels, however, CCD arrays of other dimensions may be used. In the preferred embodiment, a CCD array of 640x480 pixels is used. Using the CCD array, a "snap shot" of the OMD surface is created using known image or frame grabbing techniques, and an analog electrical representative of the snap shot is conducted to the signal processing function **348** within the system controller **346**, which includes an analog-to-digital converter, to convert the signal into an output of the data written on the OMD.

In a preferred embodiment, the detector is a commercially-available, PC-interfaceable CCD camera sold

under the trademark QuickCam™ by Connectix Corporation of San Mateo, Calif., which has a resolution of 640×480 pixels. Any other such camera may be used. The camera has a manually-adjustable focus lens, but image acquisition is otherwise controlled by the system controller **346**, which, as part of its software, initializes the camera for frame grabbing. Any such PC-interfaceable CCD camera with a similar or better resolution may be used. Other types of detector arrays known within the bar code scanning technology, including CMOS sensors, can be used in place of the CCD detector array **344**.

Processing of the image grabbed by the image detector is a significant aspect of the system in that it provides the flexibility to manipulate the image to enhance readability. The steps of the exemplary image processor are provided in the flow diagram of FIG. **31**, and the image signal generated by the detector is checked for completeness, validity and orientation, among other things. As discussed above, if systems where physical orientation and positioning of the OMD is not assured by the handling hardware, one aspect of the image processing software is to determine skew or rotation of the image as seen by the detector. The following steps are provided in detail in the system processor's software, and a portion of which is depicted in the flow diagram of FIG. **31**. [Note that the actual image obtained from the camera can be displayed on a system monitor as it is being modified to permit decoding.] First, after obtaining the image from the camera [step **1001**], in steps **1002** and **1003**, the edges of the symbol in the vertical direction are identified, looking for the highest peak signal to provide a reference, then the horizontal edges are found [step **1004**]. Knowing the boundaries of the symbol, the reasonable spacing is determined [step **1005**] to correct for missing or extra vertical edges using a neural network approach. Based on the reasonable spacing, it is determined if the length of the vertical edge is appropriate [step **1006**]; if not, adjustments are made by adding or removing edges [step **1007**]. A similar procedure is used for the horizontal edges [steps **1008–1010**], allowing skew to be determined. Having determined the orientation and spacing of the symbol, the symbol is broken into sections [step **1011**], or cells, and the average intensity for each cell is determined [step **1012**] to permit calculation of the threshold [step **1013**] for distinguishing a dark from a light area of the code. Following this, parity checks are performed [step **1014**] to provide an indication of whether the complete symbol was detected, i.e., the correct number of bits was obtained, or whether the signal was corrupted. In the preferred embodiment, 17 bits of the data contained within the two-dimensional bar code are dedicated to parity checking. At this point, if a corrupted signal is indicated, and the error is not corrected [step **1015**], rather than proceeding with an attempt to decode, the image processor initiates a new scan of the symbol [step **1001**]. If the signal is good, the cells are converted to code [step **1016**] which is decoded [step **1017**].

The image processing system converts the stored image into a series of rows [and columns for two-dimensional codes] containing binary data, with dark lines indicating highs, or ones, and white lines indicating lows, or zeroes [or vice versa]. To enhance accuracy, a number of processing steps may be performed, and the resulting data can be the average of all processing steps for that particular image.

Although efforts are taken initially to avoid modification of the surface on which the information is written, because each OMD is presumably being subjected to a significant number of process steps, the appearance of the symbol may degrade with time due to accumulation of chemicals or surface roughening, resulting in decreased contrast between the light and dark lines of the code. This issue can be addressed with software, which can compensate for the deterioration of the symbol. In a preferred embodiment, the software includes neural network algorithms which can be trained to learn the specific cumulative effects of chemical processing and compensate by either recalibrating the detector, for example, to increase the exposure time, to modify the illumination, to increase the number of verification decode steps used for averaging, or to adjust the threshold between "dark" and "light". Two appropriate commercially-available neural network programs are Thinks™ and ThinksPro™ [published by Logical Designs Consulting, Inc. La Jolla, Calif.; see, U.S. Pat. No. 5,371, 809], which are designed to run on personal computers, and provides numerous different and well known training algorithms and methods. Other neural network software products are commercially available, including Neural Works Professional™ by NeuralWare, NeuroShell2™ by Ward Systems, BrainMaker Professional™ by California Scientific, and Neural Net Tool Kit™ by Math Works. Each could be used for training of the signal processor to compensate for degraded contrast in the symbol, and selection of the appropriate program or creation of an appropriate program would be within the level of skill in the art.

FIG. **32** provides a diagram of an exemplary handling system for separating and reading and/or writing to an OMD, particularly those in the shape of a parallelopiped. Such handlers are commercially available [e.g., from Hoppmann Corporation, Chantilly, Va., see, U.S. Pat. Nos. 5,333, 716, 5,236,077, 5,145,051, 4,848,559 4,828,100, 4,821,920, 4,723,661 and 4,305,496]. The OMDs are placed in vibratory feeder **1102** by way of supply hopper **1104**. Vibratory feeder **1102** includes rings and ramps [not shown] which support the OMDs as they move within the feeder, driven by the feeder's vibration in a direction toward exit chute **1106**. An orientation rim, bar, or other feature [not shown] may be included in the internal ramps or exit chute to rotate the OMDs when a physical orientation indicator, such as the cut corner, is provided. Exit chute **1106** feeds the OMDs to ramp **1110** of linear feeder **1108**. The reciprocating motion of the ramp **1110** causes the OMDs to move forward [to the left in the figure) toward walking beam **1112** and within the field of view of camera **1114**. [Where a write operation is to be performed, the write laser and optics can be positioned in place of or nearby the camera.] Movement of the walking beam **1112** is stepped so as to pause advance motion of the OMD to allow writing and/or reading of the appropriate information.

After completion of the writing or reading step, the OMD is advanced along the walking beam **1112** toward one or more vials or flasks **1114** containing chemical or biological solutions. Ramps [not shown] leading from the walking beam to the vials or flasks **1114** can be selected by opening gates, or by tilting the walking beam **1112** in front of the selected vial, thus feeding the OMD into the desired vial for the next process step. The vials or flasks **1114** can be fixed within a tray or rack that allows it to be removed after the processing has finished so that the OMDs can be dumped into the hopper of the same or another feeder to repeat the above steps for handling, writing, reading, and distributing the OMDs to the next process step.

It may be desirable to include a protective enclosure **1116**, such as a polycarbonate and polyphenylene oxide resins, preferably the polycarbonate resin sold under the name LEXAN™ [the well known polycarbonate resin commercially available from General Electric Corp, Waterford,

N.Y., or MERLON™ made by Mobey Chemical Co., Pittsburg, Pa.] or the resin sold under the tradename NORYL [from General Electric Corp] other such polymer such as polyethylene, lucite, bakelite and other such resins that have high tensile and impact strength over a broad temperature range, are virtually shatter-proof and are extrudable as transparent sheets, over the handling system to prevent contamination of the OMDs and solutions as well as for the safety of the system operator.

2. Pre-coded memory devices

Alternatively, the matrices attached thereto may be encoded with a pre-programmed identifying bar code, such as the 2-D optical bar code that will be encoded on the matrix and read by laser. Such pre-coded devices may be used in embodiments in which parameters, such as location in an automated synthesizer, are monitored. The identity of a product or reactant determined by its location or path, which is monitored by reading the chip in each device and storing such information in a remote computer.

C. Data storage units with memory

In embodiments in which OMDs are used, the programmable devices are remote memories, such as computers into which information regarding the encoded information and linked molecules and biological particles is stored. The OMDs are read/write devices or are precoded devices.

For use with the matrices in which the memory, rather than a code, is linked to the device, any remotely programmable data storage device that can be linked to or used in proximity to the solid supports and molecules and biological particles as described herein is intended for use herein. Preferred devices are rapidly and readily programmable using penetrating electromagnetic radiation, such as radio frequency or visible light lasers, operate with relatively low power, have fast access [preferably 1 sec or less, more preferably 10^2–10^3 sec], and are remotely programmable so that information can be stored or programmed and later retrieved from a distance, as permitted by the form of the electromagnetic signal used for transmission. Presently preferred devices are on the order of 1–10 mm in the largest dimension and are remotely programmable using RF, microwave frequencies or radar [see, e.g., Roland et al. (1996) *Nature* 381:120].

Recording devices may be active, which contain a power source, such as a battery, and passive, which does not include a power source. In a passive device, which has no independent power source, the transmitter/receiver system, which transfers the data between the recording device and a host computer and which is preferably integrated on the same substrate as the memory, also supplies the power to program and retrieve the data stored in the memory. This is effected by integrating a rectifier circuit onto the substrate to convert the received signal into an operating voltage.

Alternatively, an active device can include a battery [see, e.g., U.S. Pat. No. 5,442,940, U.S. Pat. No. 5,350,645, U.S. Pat. No. 5,212,315, U.S. Pat. No. 5,029,214, U.S. Pat. No. 4,960,983] to supply the power to provide an operating voltage to the memory device. When a battery is used the memory can be an EEPROM, a DRAM, or other erasable memory requiring continuous power to retain information. It may be desirable to combine the antenna/rectifier circuit combination with a battery to create a passive/active device, with the voltages supplied by each source supplementing each other. For example, the transmitted signal could provide the voltage for writing and reading, while the battery, in addition to supplementing this voltage, provides a refresh voltage for a DRAM memory so that data is retained when the transmitted signal is removed.

The remotely programmable device can be programmed sequentially to be uniquely identifiable during and after stepwise synthesis of macromolecules or before, or during, or after selection of screened molecules. In certain embodiments herein, the data storage units are information carriers in which the functions of writing data and reading the recorded data are empowered by an electromagnetic signal generated and modulated by a remote host controller. Thus, the data storage devices are inactive, except when exposed to the appropriate electromagnetic signal. In an alternative embodiment, the devices may be optically or magnetically programmable read/write devices.

1. Electromagnetically Programmable Devices

The programmable devices intended for use herein, include any device that can record or store data. A preferred device will be remotely programmable and will be small, typically on the order of 10–20 mm^3 [or 10–20 mm in its largest dimension] or, preferably smaller. Any means for remote programming and data storage, including semiconductors and optical storage media are intended for use herein. These include Yagi antennaes [see, e.g., Roland et al. (1996) *Nature* 381:120], diodes, magnetic tapes, and any other medium for storing information.

Also intended for use herein, are commercially available precoded devices, such as identification and tracking devices for animals and merchandise, such those used with and as security systems [see, e.g., U.S. Pat. Nos. 4,652,528, 5,044,623, 5,099,226, 5,218,343, 5,323,704, 4,333,072, 4,321,069, 4,318,658, 5,121,748, 5,214,409, 5,235,326, 5,257,011 and 5,266,926], and devices used to tag animals. These devices may also be programmable using an RF signal. These device can be modified, such as by folding it, to change geometry to render them more suitable for use in the methods herein. Of particular interest herein are devices sold by BioMedic Data Systems, Inc, NJ [see, e.g., the IPTT-100 purchased from BioMedic Data Systems, Inc., Maywood, N.J.; see, also U.S. Pat. Nos. 5,422, 636, 5,420,579, 5,262,772, 5,252, 962, 5,250,962, and see, also, U.S. application Ser. No. 08/322,644, filed Oct. 13, 1994]. ID tags available from IDTAG™ Inc, particularly the IDT150 read/write transponder [ITDAG™ Ltd. Bracknell, Berks RG12 3XQ, UK, fabricated using standard procedures and the method for coil winding, bonding and packaging described in International PCT application Nos. WO95/33246, WO95/16270, WO94/24642, WO93/12513, WO92/15105, WO91/16718; see, also U.S. Pat. Nos. 5,223,851, 5,261,615 and 5,281,855] are also preferred herein. The IDT150 is a CMOS device that provides a kilobit of EEROM. This transponder also includes a 32 bit fixed code serial number that uniquely identifies each chip. The IDTAG™ transponder transmits data to a transceiver system by amplitude modulating its coil and generating an EM field. It receives data and commands from a transceiver by demodulating the field received by the coil and decoding the commands. The transponder derives its power source from a frequency emitted in the signal from the reader, to which the transponder emits a response. A smaller version [that has 16 bit EEROM] and is about 11 mm×4 mm×3 mm of this transponder is also among preferred devices. These transponders are packaged in glass or polystyrene or other such material. Also preferred herein, are tags fabricated by and available from MIKRON under the name HITAG®[see, e.g., U.S. Pat. No. 5,345,231 for a description of the systems for reading and writing].

In a preferred embodiment herein, the data storage unit includes a semi-conductor chip with integrated circuits

formed thereon including a memory and its supporting circuitry. These devices can be written to and interrogated from a distance. A radio frequency transmitter/receiver system supplies power to program and retrieve data. In particular, the data storage unit preferably includes a programmable read only semiconductor memory [PROM], preferably a non-volatile memory or other memory that can store data for future retrieval, that will have information describing or identifying the molecules or biological particles linked to or in proximity to the matrix. This information either identifies the molecule or biological particles including a phage and viral particles, bacteria, cells and fragments thereof, provides a history of the synthesis of the molecule, or provides information, such as a batch number, quality control data, reaction number, and/or identity of the linked entity. The memory is programmed, before, during or, preferably, after, each step of synthesis and can thereafter be read, thereby identifying the molecule or its components and order of addition, or process of synthesis.

While many well known read only memory devices use fuse structures that are selectively "blown" to store data points, with a fuse located at each possible data address in an array, among the devices of interest herein are those that rely on antifuse programming technology, in which short circuits are selectively created through an insulating layer separating word and bit lines in an array. Due to the relatively low level of voltage supplied by the transmitted signal when the memory device is passive, antifuse memories are readily used because of the lower voltage requirements for writing.

Thus, suitable memory devices, are about 1–20 mm in the smallest dimension [or smaller], are rapidly programmable [1 sec, preferably 1 msec or less], can be interrogated from a distance [distances of about a centimenter up to about an inch are presently preferred], and are programmable using electro-magnetic radiation, preferably frequencies, such as those within the radio frequency range, that do not alter the assessed activities and physical properties of the molecules and biological particles of interest.

Devices that rely on programmable volatile memories are also intended for use herein. For example, a battery may be used as to supply the power to provide an operating voltage to the memory device. When a battery is used the memory can be an EEPROM, a DRAM, or other erasable memory requiring continuous power to retain information. It may be advantageous to combine the antenna/rectifier circuitry with a battery to create a passive/active device, in which the voltages supplied by each source supplement each other. For example, the transmitted signal could provide the voltage for writing and reading, while the battery, in addition to supplementing this write/read voltage, provides a refresh voltage for a DRAM memory so that data is retained when the transmitted signal is removed. A 2 mmx2 mmx0.1 mm chip [or 3 mmx3 mmx0.1 mm] is presently among the preferred chips [fabricated by Sokymat]. This chip has monolithic antenna. Such chips addressable [read/write] using microwave [Ghrtz] range frequencies.

a. Antifuses

An antifuse contains a layer of antifuse material sandwiched between two conductive electrodes. The antifuse device is initially an open circuited device in its unprogrammed state and can be irreversibly converted into an essentially short circuited device by the application of a programming voltage across the two electrodes to disrupt the antifuse material and create a low resistance current path between the two electrodes.

An exemplary antifuse structure for use herein is formed by defining a word line of heavily N-doped polysilicon on an insulating substrate, depositing an antifuse layer of lightly N-doped semiconductor over the polysilicon, and defining a metal address [or bit] line upon and in electrical contact with the antifuse layer. The semiconductor material used for the antifuse layer is typically selected from among silicon, germanium, carbon and alpha-tin. The properties of the semiconductor material are such that the material is essentially non-conductive as long as the voltage across it does not exceed a threshold level. Once the threshold voltage is exceeded, a conductive filament is formed through the semiconductor so that the resistance between the metal and polysilicon lines at the points at which they cross irreversibly switches from a high resistance state to a relatively low resistance state.

To program or change the resistance of the antifuse from a very high level [greater than 100,000,000 ohms] to a low level [less than 1000 ohms], a voltage of sufficiently high electrical field strength is placed across the antifuse film to create a short circuit. The voltage level required to induce breakdown is determined by the level of dopant in the antifuse layer. As breakdown occurs electrical current will flow through one small region of the film. The current is limited by the resistance of the filament itself as well as any series resistance of conductive layers or logic devices [transistors] in series with the antifuse.

Examples of the antifuse and its use as a memory cell within a Read-Only Memory are discussed in Roesner et al., "Apparatus and Method of Use of Radio frequency Identification Tags", U.S. application Ser. No. 08/379,923, filed Jan. 27, 1995, Roesner, "Method of Fabricating a High Density Programmable Read-Only Memory", U.S. Pat. No. 4,796,074 (1989) and Roesner, "Electrically Programmable Read-Only Memory Stacked above a Semiconductor Substrate", U.S. Pat. No. 4,442,507 (1984). A preferred antifuse is described in U.S. Pat. No. 5,095,362. "Method for reducing resistance for programmed antifuse" (1992) [see, also U.S. Pat. Nos. 5,412,593 and 5,384,481].

U.S. Pat. No. 5,095,362 provides a method for fabricating a layer of programmable material within an antifuse that exhibits relatively lower than normal resistance in its programmed state and also provides a semiconductor device containing an antifuse film of the type composed of semiconductor material having a first electrical state that is characterized by high electrical resistivity and a second electrical state that is characterized by low electrical resistivity.

The means for selectively decreasing resistivity includes nonactivated conductive dopants that are ion implanted within the otherwise highly resistive semiconductor material. The dopants as implanted are in a nonactivated state so that the dopants do not enhance the conduction of carriers in the film. Once activated, the dopants enhance the conduction of carriers in the film. Activation of the dopants occurs upon application of a threshold voltage across a predetermined and selected portion of the material in which the dopants are disposed. The selected portion is defined by the crossover point of selected word and bit [or address lines. The dopants are N-type, selected from among antimony, phosphorous, arsenic, and others to provide additional charge carriers. The implant dosage is used to determine the threshold voltage level that will be required to induce formation of the conductive filament. P-type dopants, such as boron, may also be used to affect a change in programming voltage.

b. A recording device with non-volatile memory

FIG. 5 depicts a recording device containing a non-volatile electrically-programmable read-only memory

(ROM) **56** that utilizes antifuse, EEPROM or other suitable memory, is combined on a single substrate **54** with a thin-film planar antenna **60** for receiving/transmitting an RF signal **58**, a rectifier **62** for deriving a voltage from a received radio frequency (RF) signal, an analog-to-digital converter (ADC) **64** for converting the voltage into a digital signal for storage of data in the memory, and a digital-to-analog converter (DAC) **66** for converting the digital data into a voltage signal for transmission back to the host computer is provided. A single substrate **54** is preferred to provide the smallest possible chip, and to facilitate encapsulation of the chip with a protective, polymer shell (or shell plus matrix or matrix material) **52**. Shell **52** must be non-reactive with and impervious to the various processes that the recording device is being used to track in order to assure the integrity of the memory device components on the chip. Materials for the shell include any such materials that are known to those of skill in the art, including glasses, ceramics, plastics and other inert coatings.

Based on current semiconductor integrated circuit fabrication process capabilities, in a preferred embodiment the finished chip on which all of the listed components are integrated is on the order of 1 mm×1 mm [~40 mils×40 mils], with a memory capacity of about 1024 bits, but can have greater or lesser capacity as required or desired. Greater memory capacity, where needed, and smaller chips, however, will be preferred.

The chip may be larger to accommodate more memory if desired, or may be smaller as design rules permit smaller transistors and higher device densities, i.e., greater memory capacity.

The antifuse ROM structure described herein, and the method for fabricating the same, are based upon the known devices. (See, e.g., U.S. Pat. No. 4,424,579, No. 4,442,507, No. 4,796,074, No. 5,095,362, No. 4,598,386, No. 5,148, 256, No. 5,296,722, and No. 5,583,819.)

In an antifuse-type memory device, the individual memory cells are arranged in arrays of orthogonal conductive word and bit lines to obtain the smallest possible memory array size. For example, for 1024 bits of memory, there are 32 word lines and 32 bit lines for a square array. Memories with greater capacity may also be used. Schottky diodes are formed generally corresponding to the points at which the word and bit lines cross. The word and bit lines are separated by an undoped or lightly-doped semiconductor layer with interstitial doping. The semiconductor layer may also be amorphous silicon with implanted dopants in a nonactivated state. Each of these crossover points is a memory cell and is the equivalent of a programmable switch in series with a Schottky diode. Data are stored by the switch being ON or OFF. As fabricated, an antifuse memory device has all of its switches in the OFF state. A switch is turned on by applying a voltage in excess of a pre-determined threshold voltage to one of the word lines while setting a selected bit line to a low logic level. The threshold voltage is determined by the impedance of the semiconductor layer, i.e., its doping level. According to the process for fabricating the antifuse memory of the preferred embodiment, the impedance can be less than 200 ohms with a threshold voltage for programming as low as 3 volts. Since in the embodiment described herein the programming voltage is provided solely by the rectified RF signal, a low threshold is preferred. Application of voltage exceeding the threshold activates the interstitial dopant in the semiconducting film at the point corresponding to the cross-over between the two lines, causing a short between the word and bit lines and irreversibly turning on that particular switch or memory cell. Address decoders, as are known in the art, are used to selectively address the word and bit lines for purposes of writing information to and reading stored information from the memory array. [See, e.g., U.S. Pat. No. 5,033,623, 5,099,226, 5,105,190, 5,218,343, 5,323,7041. Exemplary means for decoding information to be stored in memory and to be read from memory are provided in U.S. Pat. Nos. 4,442,507 and No. 4,598,386.

Information to be written into the memory need not be detailed since the data stored in the memory is primarily acting as an identification marker that is traceable to a more detailed record stored in the host computer memory **68**, independent of the memory associated with the matrix support or tagged molecule or biological particle. In this manner, the RF signal from transmitter **50** that is used to provide the power and the signal to the matrix particle memory need only address a single memory cell to indicate that a nascent oligomer linked to or in proximity to the memory device has been subjected to a given process step or to identify a molecule or biological particle. Thus, a sophisticated memory addressing system need not be provided on the matrix particle memory chip, and shift registers may be used to control memory addressing. Alternatively, a microprocessor which is mask-programmed during the fabrication process for controlling an address bus which connects the ADC **64** and the DAC **66** to the memory array may also be built onto the same substrate on which the memory and other components are integrated. Other integrated means for selectively addressing locations within the memory are known and will be apparent to the practitioner skilled in the art.

As described above, antifuse memories are well known in the art. These memories include structures in which the word and bit lines may be made of either N+polysilicon or metal [aluminum or aluminum-silicon], separated by silicon dioxide (SiO_2), silicon nitride (Si_3N_4), combinations thereof, or amorphous silicon alone or in combination with SiO_2 and/or Si_3N_4. In each case, a short circuit is created at locations in the antifuse material corresponding to the crossover location of selected word and bit lines by applying a voltage in excess of a pre-determined threshold voltage.

Examples of alternate means for forming an antifuse memory are provided in the following U.S. Pat. No. 5,248,632, issued Sep. 28, 1993, of Tung et al.; U.S. Pat. No. 5,250,459, issued Oct. 5, 1993, of Lee, No. 5,282,158, issued Jan. 25, 1994, of Lee; No. 5,290,734, issued Mar. 1, 1994, of Boardman, et al.; U.S. Pat. No. 5,300,456, issued Apr. 5, 1994, of Tigelaar et al.; U.S. Pat. No. 5,311,039, issued May 10, 1994, of Kimura, et al.; U.S. Pat. No. 5,316,971, issued May 31, 1994, of Chiang et al.; U.S. Pat. No. 5,322,812, issued Jun. 21, 1994, of Dixit, et al.; U.S. Pat. No. 5,334,880, issued Aug. 2, 1994, of Abadeer, et al., and others.

Generally for use in the methods herein, non-volatility of the memory or the ability to lock or prevent erasure is preferred since power is applied to the chip only when it is subjected to the RF or other transmission signal for reading or reading and writing. Further considerations are the voltage levels required for writing into memory, since the threshold voltage must be less than the maximum voltage of the rectified RF signal in order to assure that sufficient voltage is always available during the writing process. The write voltage may be enhanced by supplementing the RF-supplied voltage with optically-generated voltage, such as a photocell. Photocells on semiconductor substrates are well known in the art and could be easily integrated onto the chip. A laser or other light source could be readily included

in the write apparatus to illuminate the chip coincident with transmission of the RF write signal. Similarly, other forms of electromagnetic radiation may be used to provide additional power, if needed.

Although antifuse memories are not designed to be erasable, it may be desirable to re-use the devices if the memory becomes full. In such instances, conventional electrically programmable erasable read only memories [EEPROMs] may be used instead. Since EEPROMs require higher write voltage levels, it may be desirable to supplement the RF-supplied voltage as described above. In EEPROMs, stored data can be erased by exposing the device to UV light.

Signal rectifier **62** may be one or more Schottky diode(s), making it readily incorporated into the fabrication process used for the memory array. Other means for signal rectification may be used as are known. The ADC **64** and DAC **66** are well-known devices and are readily integrated onto the substrate **54** using the fabrication process described in the references for the memory array. Radio frequency modulation techniques, which are known in the art, for example, pulse code modulation, may be adapted to permit direct digital transmission, in which case the ADC and DAC may not be required.

Antenna **60** is formed during the fabrication process using conventional photolithographic techniques to provide one or more metal structures, such as aluminum, to receive a pre-determined wavelength RF transmission. The antenna may be a simple straight line half-wave antenna which is created by patterning a structure during the second metal process steps so that the structure has a length equal to one-half of the wavelength of the selected RF transmission frequency in free space. Another option for formation of the antenna is as a small loop, either on a dedicated portion of the chip, or encircling the other components of the chip, also formed during the second metal step of the fabrication process. It is noted that, in a typical semiconductor fabrication process, such as would be compatible with the preferred antifuse memory, the first and second metal steps include depositing a layer of aluminum, then patterning the aluminum photolithographically followed by a plasma etch to define the desired features. Except where vias are formed, the two metal layers are separated by a dielectric film. Dipole antennas may be formed by patterning the second metal in a similar manner, with the dimensions of the antenna being selected for the appropriate RF frequency. The two metal layers may also be used to form a microstrip antenna structure by selecting the dielectric film between the metal layers such that it has a dielectric constant and thickness appropriate so that the microstrip is resonant at one-half of the RF wavelength. (The first metal layer provides the ground plane.) The metal structures, which may be square patches, circles, lines, or other geometries, are defined photolithographically during the normal masking steps of the first and second metal processes. Other antenna structures which can be configured as a thin film device for integration onto a common substrate with the memory structure and other components may be used and will be apparent to those skilled in the art. Similarly, a resonant circuit (inductor-capacitor) can be readily integrated onto the chip, with the resonant circuit being tuned to the RF carrier signal of the transmitter.

Frequency tuning of either an antenna or resonant circuit can provide additional coding capability. For example, a first group of memory devices can be tuned to receive a carrier wave of a first RF frequency, e.g., f_1, and a second group could be tuned to receive a second frequency f_2, and so on. The separate carrier frequencies could provide additional means for tracking or providing information to the devices, even if the groups become intermixed.

The RF antenna may, in an alternate embodiment, be formed external to the semiconductor substrate. In this configuration, a separate conductive wire, which acts as an antenna, will be attached to a bond pad formed on the chip using methods known to those skilled in the art. The wire will then be stabilized when the chip is encased in the protective shell, so that the antenna extends at some angle to the chip.

Also, as an alternative to signal transmission via RF, the antifuse or other semiconductor memory and supporting circuitry can receive the addressing commands and device power by optical transmission. In this embodiment, the RF antenna **60** would be replaced by a photocell that generates sufficient write voltage to exceed the threshold voltage. For the addressing commands, the RF transmitter **50** is replaced by a light source, and the commands may be transmitted digitally by pulsing the optical transmitter, which can be a laser, flash lamp or other high intensity light source. It is noted that the light intensity must be sufficient to generate adequate voltage, either singly or in conjunction with a second power generating device, in the photocell to write into memory, but not so high that it damages the metal interconnect on the chip. With digital data transmission analog-to-digital and digital-to-analog conversion circuitry can be eliminated.

c. Other Memory Devices

Other types of electrically-programmable read-only memories, preferably non-volatile memories, which are known in the art, may be used [see, e.g., U.S. Pat. No. 5,335,219]. Chips, such as those sold by Actel, Mosaic, Lattice Semiconductor, AVID, Anicare, Destron, Rayethon, Altera, ICT, Xilinix, Intel and Signetics [see, e.g., U.S. Pat. Nos. 4,652,528, 5,044,623, 5,099,226, 5,218,343, 5,323,704, 4,333,072, 4,321,069, 4,318,658, 5,121,748, 5,214,409, 5,235,326, 5,257,011 and 5,266,926] may be used herein. Preprogrammed remotely addressable identification tags, such as those used for tracking objects or animals [see, e.g., U.S. Pat. Nos. 5,257,011, 5,235,326, 5,226,926, 5,214,409, 4,333,072, available from AVID, Norco, Calif.; see, also U.S. Pat. Nos. 5,218,189, 5,416,486, 4,952,928, 5,359,250] and remotely writable versions thereof are also contemplated for use herein. Preprogrammed tags may be used in embodiments, such as those in which tracking of linked molecules is desired. Devices sold by XCI [San Jose, Calif.] that operate in the lower frequency [~900 mhz] range are also preferred herein.

d. Pre-coded Memory Devices

Alternatively, the matrices or strips attached thereto may be encoded with a pre-programmed identifying bar code, such as an optical bar code that will be encoded on the matrix and read by laser. Such pre-coded devices may be used in embodiments in which parameters, such as location in an automated synthesizer, are monitored. The identity of a product or reactant determined by its location or path, which is monitored by reading the chip in each device and storing such information in a remote computer. Read/write tags such as the IPTT-100 [BioMedic Data Systems, Inc., Maywood, N.J.; see, also U.S. Pat. Nos. 5,422,636, 5,420,579, 5,262,772, 5,252,962, 5,250,962, and U.S. application Ser. No. 08/322,644] are also contemplated for use herein.

Among the particularly preferred devices are the chips [particularly, the IPTT-100, Bio Medic Data Systems, Inc.,

Maywood, N.J.; see, also U.S. Pat. Nos. 5,422,636, 5,420,579, 5,262,772, 5,252,962 and 5,250,962 and U.S. application Ser. No. 08/322,644,] that can be remotely encoded and remotely read. These devices, such as the IPTT-100 transponders that are about 8 mm long, include a recording device, an EEPROM, a passive transponder for receiving an input signal and transmitting an output signal in response. In some embodiments here, the devices are modified for use herein by altering the geometry. They are folded in half and the antenna wrapped around the resulting folded structure. This permits convenient insertion into the microvessels and formation of other combinations.

These devices include a power antenna means [see, e.g., U.S. Pat. No. 5,250,944 and U.S. Pat. No. 5,420,579] for receiving the input signal, frequency generator and modulator means for receiving the input signal the receive antenna means and for generating the output signal. The output signal has a frequency different from the input frequency, outputs the output signal in response the input signal. The input signal having a first frequency, the output signal has a second frequency that is a multiple of the first frequency, and is greater that the first frequency. It also includes a transmitting antenna means for receiving the output signal from the frequency generator and modulator means and that transmit the output signal. Data are stored within the transponder within a reprogrammable memory circuit that is programmed by the user [see, e.g., U.S. Pat. No. 5,422,636 and EP 0 526 173 A3]. A transponder scanner for scanning and programming the transponder is also available [Bio Medic Data Systems Inc. DAS-5001 CONSOLE™ System, e.g., U.S. Pat. No. 5,252,962 and U.S. Pat. No. 5,262,772].

e. Other Memories

Another such device is a 4 mm chip with an onboard antenna and an EEPROM [Dimensional Technology International, Germany]. This device can also be written to and read from remotely.

ID tags available from IDTAG™ Inc, particularly the IDT150 read/write transponder [ITDAG™ Ltd. Bracknell, Berks RG12 3XQ, UK], discussed above, are also preferred herein.

f. Monolithic Semiconductors

Additionally, smaller [about 2 mmx2 mmx0.1 mm or less] monolithic devices are of interest herein. For example, in a particular embodiment of an electromagnetically programmable tag, such as, for exemplification purposes an RF tag, a single chip tag is formed entirely on a single substrate. More specifically, referring to FIG. **51**, a monolithic tag, such as an RF tag, is shown and generally designated **4700**. This monolithic tag is sized such that the substrate has following overall external dimensions: width **4710** of 2 mm wide, length **4708** of 2 mm, and a height **4714** of 0.1 mm. As a result of this miniaturization of the tag, a variety of shapes and sizes of the tags may be created [see, e.g., U.S. Pat. No. 4,857,893 issued to Carroll in 1989, entitled "Single Chip Transponder Device", which describes a single substrate (monolithic) RF transponder which, due to its single substrate, is simple to manufacture].

In FIG. **51**, the tag **4700** is shown having a substrate **4702** is formed with an antenna **4714**. This antenna is preferably formed on the substrate using a metalization process wherein a metal is placed on a pattern on the top surface of the substrate to create a particular antenna. As shown, the antenna is substantially square, tracing out a coiled antenna beginning at pad **4712**, and ending at conductor **4706** which attaches back to the circuitry **4704**. It should be appreciated that while the antenna is shown to be square, any other shapes could be used. In particular, a circular antenna could be formed just as easily on the surface of the substrate. It should be appreciated, however, that the functionality of the antenna are likely very similar between a square antenna and a circular antenna. In addition to the antenna as shown, there may be a second antenna on the back side of the substrate (not shown) which could be used to increase the number of windings or, as an alternative, be tuned for a different frequency range than the antenna patterned on the upper surface of the substrate **4702**.

Referring to FIG. **52**, the tag is shown in plan view and has circuitry **4704** which includes specific logic and control electronics generally denoted **4706**. The pattern of the antenna **4714** is easily appreciated from this view. Moreover, the circular equivalent can be easily envisioned on the substrate **4702** to spiral around the circuitry **4704** to create a similarly sized antenna.

The specific electronic circuitry that is contained in circuitry **4704** is known and well described [see, e.g., U.S. Pat. No. 4,857,893, and U.S. Pat. No. 5,345,231, discussed above] For example, U.S. Pat. No. 5,345,231, entitled "Contactless Inductive Data-Transmission System" discloses circuitry capable of communicating identification information across a wireless communication system which employs an inductive coupling. Specifically, with reference to FIG. **2** of that reference, this inductive coupling provides the power to run the tag electronics, as well as provides the communication channel with which the identification information travels. The circuitry includes a rectifier attached to the antenna to receive an electromagnetically coupled signal, and to create its own power from the signal. In addition to the rectifier, a clock extractor and demodulator also receive the antenna's signal. The clock extractor recreates a communication clock, and the demodulator decodes the signal received from the antenna using that clocking information. This information is provided to a control unit which either programs or downloads the contents of a memory bank. In the particular tag discussed herein, the memory bank can include a single data bit, or may be easily expanded as is generally known in the art to a variety of memory sizes, up to several kilobytes. Once the memory has been accessed, the control unit can communicate with the base transmitter/receiver by sending data to the modulator which is also electrically connected to the antenna. In that manner, the antenna can be used to either receive a signal from the transmitter, or to transmit a signal to the receiver.

The antenna for use with these particular electronics is tuned for a resonant frequency of approximately 125 kHz. It is to be appreciated, as discussed above, that the antenna can easily be tuned to receive a variety of frequencies, including 125 kHz. It should be noted, however, that depending on the frequencies to be received, the antenna shape and size could be altered. In fact, it should be appreciated that a single antenna which is capable of receiving a number of frequencies could be created by having electrical leads which attached to a number of points along the length of the antenna. As a result, a single antenna could be used to receive a number of frequencies, where the frequency to be received could be set by an initial communication with the tag. Because of such frequency specification, it should be appreciated that the a number of tags could be addressed simultaneously in a "batch" read or write process.

A batch read/write process enables a single transmitter station to address more than one tag at the same time. This is particularly useful when desiring to program a number of

tags with the same or similar information. Thus, by batch writing the information to a number of tags, the programming process is shortened and simplified, while at the same time, minimizing the opportunity for error.

In addition to the ease and accuracy of batch accessing the tags, such a feature is particularly useful when faced with a large number of tags to identify. More particularly, the ability to batch access the tags permits the transmitter/receiver to identify any number of tags within an area simply by one access process instead of having to access each tag individually. It should be appreciated that in addition to simply accessing all tags at one time, there could be a variety of signal types which could narrow the field of access. One manner of restricting the access to tags, such as an RF tag, could be to make any interrogation either code or frequency discriminating. Such discrimination would occur, for example, by selecting only the particular RF tags within a frequency range. On the other hand, the specific address code of a number of RF tags could be programmed such that by identifying, for example, the first four of an eight bit address scheme, only a portion of the RF tags identifiable with those eight addressing bits could be addressed.

The circuitry for communication with a transmitting and receiving base is known [see, e.g., U.S. Pat. No. 4,857,893, entitled "Single Chip Transponder Device", see esp. FIG. **2** of that reference]. Briefly, an antenna receives a carrier signal which is provided to a rectifier and demodulator, as well as a timing decoder. The rectifier captures a portion of the carrier signal to derive dc power to drive the RF tag itself. The timing decoder uses the remaining portion of the antenna signal to derive the timing signals necessary to control the data storage and generation of the RF tag. Once accessed, the data generator creates a data signal which is modulated and supplied to the antenna for retransmit back to the transmitter/receiver unit.

Referring now to FIG. **53**, the single substrate tag shown in FIGS. **51** and **52** is shown attached to a stirring bar, which is commonly known in the industry. The stirring bar includes a material which is capable of magnetic interaction. This material is then encapsulated in an inert material such as a polymer which insulates the material from the environment. As shown partially cut away for clarity, the insulation material **4718** covers the entire outer surface of the material **4716**. Prior to encapsulation, the monolithic RF tag **4700** is attached to the material such that, once encapsulated, the RF tag is also encapsulated.

Once a stirring bar is equipped with an RF tag, the stirring bar may be placed in a container and the container may then be easily tracked through any environment simply by placing the container over an identification station. Moreover, by placing a number of stirring bars in different containers, with each stirring bar having its own identification number, virtually an unlimited number of containers may be tracked. This would be particularly useful in environments where there is a need to manipulate a large number of containers, such as a biomedical laboratory. It should be appreciated, however, that in addition to placing the monolithic RF tag on a stirring bar, the tag may be attached to any other commonly used devices to facilitate tracking those devices. For instance, as described elsewhere herein, each container may be manufactured with the monolithic tags embedded in the container or in sleeve that is removable attached to a container. This permits tracking of the container throughout its environment, without the need to add any device or item to the contents of the container.

In addition to allowing the simple tracking of various containers, it is possible to place a plurality of tags, such as different RF tags, within a container that is already tagged. More specifically, it is possible to place a tag in a container that is already identified with one tag. This combining of multiple tags would enable a greater level of tracking of the container. For instance, a beaker could be formed with a RF tag integral to its structure. Once identified, the beaker may be filled with a variety of materials, each having its own identification number. Thus, when solution B is added to beaker A,. an RF tag indicating the material B can simply be dropped into the beaker A. Likewise, when solution C is added to beaker A, an RF tag indicating the material C can be dropped in the beaker. As a result of this marking method, it would be possible to verify the exact contents of a container. Specifically, by reading the various RF tags within the beaker A (solution B and solution C) the entire contents of the container would be identified.

Alternatively, in addition to, or instead of, simply identifying the contents of a container, it would also be possible to track the whereabouts of a container by adding identifying RF tags at various locations in its path. For instance, a beaker could be marked with an identifying RF tag A, and an identifying RF tag B could be added when a particular process is performed on the contents of the beaker A. Similarly, an identifying RF tag C could be added to the beaker at the next process step. Thus, the RF tags in the beaker would indicate the exact historical location of the container A simply by decoding the contents of the RF tags contained therein.

In addition, the RF tags could be used to provide a combination of the above described contents and location based information. This combination would provide a means to analyze a "chain of possession" for various contents of a container. In other words, the contents of a beaker could be determined by identifying the RF tags. This information would provide a history of the contents of the container, as well as location of the processes which were performed on the container.

2. Optically or Magnetically Programmed Devices

In addition to electrically-programmable means for storing information on the matrix particles, and the 2-D bar codes, described above, other optical and magnetic means may be used. Such optical storage means are known [see, e.g., U.S. Pat. No. 5,136,572, issued Aug. 4, 1992, to Bradley]. Here, an array of stabilized diode lasers emits fixed wavelengths, each laser emitting light at a different wavelength. Alternatively, a tunable diode laser or a tunable dye laser, each of which is capable of emitting light across a relatively wide band of wavelengths, may be used. The recording medium is photochemically active so that exposure to laser light of the appropriate wavelength will form spectral holes.

As illustrated In FIG. **8**, an optical write/read system is configured similar to that of the embodiment of FIG. **7**, with a vessel **164** containing a number of the particles which are separated and oriented by passing through a constricted outlet into a write/read path **158** that has an optically-transparent tube (i.e., optically transparent to the required wavelength(s)) with a cross-section which orients the particles as required to expose the memory surface to the laser **152** which is capable of emitting a plurality of discrete, stable wavelengths. Gating and detection similar to that described for the previous embodiment may be used and are not shown. Computer **154** controls the tuning of laser **152** so that it emits light at a unique wavelength to record a data point. Memory within computer **154** stores a record indi-

cating which process step corresponds to which wavelength. For example, for process A, wavelength λ_1, e.g., 630 nm (red), for process C, λ_2, e.g., 550 nm (yellow), and for process E, λ_3, e.g., 480 nm (blue), etc. The recording medium **156** is configured to permit orientation to repeatably expose the recording side of the medium to the laser beam each time it passes through tube **158**. One possible configuration, as illustrated here, is a disc.

To write onto the recording medium **156**, the laser **152** emits light of the selected wavelength to form a spectral hole in the medium. The light is focused by lens **160** to illuminate a spot on recording medium **156**. The laser power must be sufficient to form the spectral hole. For reading, the same wavelength is selected at a lower power. Only this wavelength will pass through the spectral hole, where it is detected by detector **162**, which provides a signal to computer **154** indicative of the recorded wavelength. Because different wavelengths are used, multiple spectral holes can be superimposed so that the recording medium can be very small for purposes of tagging. To provide an analogy to the electrical memory embodiments, each different wavelength of light corresponds to an address, so that each laser writes one bit of data. If a large number of different steps are to be performed for which each requires a unique data point, the recording media will need to be sufficiently sensitive, and the lasers well-stability, to vary one within a narrow band to assure that each bit recorded in the media is distinguishable. Since only a single bit of information is required to tag the particle at any given step, the creation of a single spectral hole at a specific wavelength is capable of providing all of the information needed. The host computer then makes a record associating the process performed with a particular laser wavelength.

For reading, the same wavelength laser that was used to create the spectral hole will be the only light transmitted through the hole. Since the spectral holes cannot be altered except by a laser having sufficient power to create additional holes, this type of memory is effectively non-volatile. Further, the recording medium itself does not have any operations occurring within its structure, as is the case in electrical memories, so its structure is quite simple. Since the recording medium is photochemically active, it must be well encased within an optically transmissive [to the active optical wavelength(s)], inert material to prevent reaction with the various processing substances while still permitting the laser light to impinge upon the medium. In many cases, the photochemical recording media may be erased by exposure to broad spectrum light, allowing the memory to be reused.

Writing techniques can also include the formation of pits in the medium. To read these pits, the detector **162** with be positioned on the same side of the write/read tube **158** as the laser **152** to detect light reflected back from the medium. Other types of optical data storage and recording media may be used as are known in the art. For example, optical discs, which are typically plastic-encapsulated metals, such as aluminum, may be miniaturized, and written to and read from using conventional optical disc technology. In such a system, the miniature discs must be aligned in a planar fashion to permit writing and reading. Other optical recording media that may be appropriate for use in the recording devices and combinations herein include, but are not limited to, magneto-optical materials, which provide the advantage of erasability, photochromic materials, photoferroelectric materials, photoconductive electro-optic materials, all of which utilize polarized light for writing and/or reading, as is known in the art. When using any form of optical recording, however, considerations must be made to insure that the selected wavelength of light will not affect or interfere with reactions of the molecules or biological particles linked to or in proximity to matrix particles.

a. Three Dimensional Optical Memories

3-D memory storage devices include persistent hole burning, phase holograms, and two photon optical 3-D memories that use organic materials and biomolecules. Any such devices are intended for use herein. Of particular interest are those that use organic materials and biomolecules. Such memories can be incorporated into the matrix materials or inert polymeric materials that are derivatized for use as matrices.

b. 3-D Optical Memories and Apparatus Therefor

Optical memory systems are based on light-induced changes in the optical chemical or physical properties of materials. As such these memories are ideally suited for use in the methods herein and in combination with matrices, since the materials that form the memory may be incorporated into or part of the material from which the matrix is fabricated.

Polymer-based photonic materials that can store 1 trillion bytes of date per cc have been developed [see, e.g., U.S. Pat. Nos. 5,268,862, 5,130,362, 5,325,324; see, also, Dvornikov et al. (1996) *Opt. Commun.* 128:205–210; Dvornikov et al. (1996) *Res. Chem. Intermed.* 22:115–28; Dvornikov et al. (1994) *Proc. SPIE-Int. Soc. Ort. Eng.* 2297:447–51; Dvornikov et al. (1994) *Mol. Cryst. Liq. Cryst. Sci. Technol., Sect. A* 246:379–88; Dvornikov et al. (1994) *J. Phys. Chem.* 98:6746–52; Ford et al. (1993) *Proc. SPIE-Int. Soc. Opt.* 2026:604–613; Ford et al. *Proc. SPIE-Int. Soc. Ort. Eng.* 1853:5–13; Malkin et al. *Res. Chem. Intermed.* 19:159–89; Dvornikov et al. (1993) *Proc. SPIE-Int. Soc. Opt. Eng.* 1852:243–52; Dvornikov et al. (1992) *Proc. SPIE-Int. Soc. Opt. Eng.* 1662:197–204; Prasad et al. (1996) *Mater. Res. Soc. Symp. Proc.* 413:203–213; and Dagani in *Chemical and Eng. News* Sep. 23, 1996, pp. 68–69]. This technology involves using a laser to encode information in a polymeric medium containing dye molecules that have a nonlinear optical property known to those of skill in the art as two-photon absorption. When the dye molecule is irradiated with light of sufficiently high intensity i, it absorbs two photons of light simultaneously; the molecule then emits a photon of higher energy. This means that the material can be irradiated with lower energy penetrating light, such infrared or near infrared and produce a higher energy emission in the visible.

In these methods, the writing beam "photobleaches" spots in the recording medium so that those spots when subsequently illuminated with a reading beam will emit either no light or less light than the surrounding medium [see, e.g., U.S. Pat. No. 5,325,324; see, also U.S. Pat. No. 5,130,362]. By varying the intensity of the writing laser, the extent of photobleaching can be varied to get a gray-scale, thereby permitting storage of information in analog as well as digital form. Dyes have been developed that are particularly suitable for use in these methods. For example, a stilbene derivative with substituted amino and sulfonyl groups [APSS] has been developed that has very strong 2-photon absorption. The dye is dispersed in a polymer, such as a methacrylate polymer, which then serves as a read/write medium.

Memories based on photochromaic materials, such as 1-nitro-2-naphthaldehyde and the colorless base form of the

laser dye rhodamine B, are also available [see, e.g., Dvornikov et al. (1996) *Res. Chem. Intermed.* 22:115–28].

As noted above, incorporation of the these dyes or other such molecules into the polymeric supports used in the syntheses and assays described herein will permit the supports [or portions thereof] to serve as memories to which information can be written and from which it can be read. Thus, for example, instead of using a symbology as described in the embodiments herein, the polymer from which support is made will contain a dye molecule or other molecule that exhibits 2-photon absorption, and thereby serve as a storage medium that can be read or can be a read/write medium. The other portion of the device can be radiation grafted and used as a support for chemical syntheses and assays.

c. Rhodopsins

Another memory means that is suitable for use in the matrix with memory combinations are optical memories that employ rhodopsins, particularly bacteriorhodopsin [BR], or other photochromic substances that change between two light absorbing states in response to light of each of two wavelengths [see, e.g., U.S. Pat. Nos. 5,346,789, 5,253,198 and 5,228,001; see, also Birge (1990) *Ann. Rev. Phys. Chem* 41:683–733]. These substances, particularly BR, exhibit useful photochromic and optoelectrical properties. BR, for example, has extremely large optical nonlinearities, and is capable of producing photoinduced electrical signals whose polarity depends on the prior exposure of the material to light of various wavelengths as well as on the wavelength of the light used to induce the signal. There properties are useful for information storage and computation. Numerous applications of this material have been designed, including its use as an ultrafast photosignal detector, its use for dynamic holographic recording, and its use for data storage, which is of interest herein.

The rhodopsins include the visual rhodopsins, which are responsible for the conversion of light into nerve impulses in the image resolving eyes of mollusks, anthropods, and vertebrates, and also bacteriorhodopsin [BR]. These proteins also include a class of proteins that serve photosynthetic and phototactic functions. The best known BR is the only protein found in nature in a crystalline membrane, called the "purple membrane" of *Halobacterium Halobium*. This membrane converts light into energy via photon-activated transmembrane proton pumping. Upon the absorption of light, the BR molecule undergoes several structural transformations in a well-defined photocycle in which energy is stored in a proton gradient formed upon absorption of light energy. This proton gradient is subsequently utilized to synthesize energy-rich ATP.

The structural changes that occur in the process of light-induced proton pumping of BR are reflected in alterations of the absorption spectra of the molecule. These changes are cyclic, and under usual physiological conditions bring the molecule back to its initial BR state after the absorption of light in about 10 milliseconds. In less than a picosecond after BR absorbs a photon, the BR produces an intermediate, known as the "J" state, which has a red-shifted absorption maximum. This is the only light-driven event in the photocycle; the rest of the steps are thermally driven processes that occur naturally. The first form, or state, following the photon-induced step is called "K", which represents the first form of light-activated BR that can be stabilized by reducing the temperature to 90° K. This form occurs about 3 picoseconds after the J intermediate at room temperature. Two microseconds later there occurs an "L" intermediate state which is, in turn, followed in 50 microseconds by an "M" intermediate state.

There are two important properties associated with all of the intermediate states of this material. The first is their ability to be photochemically converted back to the basic BR state. Under conditions where a particular intermediate is made stable, illumination with light at a wavelength corresponding to the absorption of the intermediate state in question results in regeneration of the BR state. In addition, the BR state and intermediates exhibit large two-photon absorption processes which can be used to induce interconversions among different states.

The second important property is light-induced vectorial charge transport within the molecule. In an oriented BR film, such a charge transport can be detected as an electric signal. The electrical polarity of the signal depends on the physical orientation of molecules within the material as well as on the photochemical reaction induced. The latter effect is due to the dependence of charge transport direction on which intermediates [including the BR state] are involved in the photochemical reaction of interest. For example, the polarity of an electrical signal associated with one BR photochemical reaction is opposite to that associated with a second BR photochemical reaction. The latter reaction can be induced by light with a wavelength around 412 nm and is completed in 200 ns.

In addition to the large quantum yields and distinct absorptions of BR and M, the BR molecule [and purple membrane] has several intrinsic properties of importance in optics. First, this molecule exhibits a large two-photon absorption cross section. Second, the crystalline nature and adaptation to high salt environments makes the purple membrane very resistant to degeneration by environmental perturbations and thus, unlike other biological materials, it does not require special storage. Dry films of purple membrane have been stored for several years without degradation. Furthermore, the molecule is very resistant to photochemical degradation.

Thus, numerous optical devices, including recording devices have been designed that use BR or other rhodopsin as the recording medium [see, e.g., U.S. Pat. Nos. 5,346,789, 5,253,198 and 5,228,001; see, also Birge (1990) *Ann. Rev. Phys. Chem* 41:683–733]. Such recording devices may be employed in the methods and combinations provided herein.

3. Event-detecting Embodiment and Sensors

Combinations of the matrices with memories and sensors, such as biosensors and devices that measure external parameters are provided. Combinations of memories with sensors are also provided. Various embodiments of the sensors are set forth in the Examples. See, also FIG. 9 and the description below. Examples of glucose sensors, calcium sensors, urea sensors, and intracranial pressure monitors are provided herein. In each embodiment, a memory is included as a means to track patient history and/or to store or record sensed information.

In particular, the memories and memories with matrices provided herein may be advantageously used in combination with sensors, which are devices that measure external parameters, such as pH, temperature, ion concentrations in solutions, and also biosensors, particularly implantable biosensors, which are used to measure internal parameters, such as electrolytes, blood glucose, to monitor blood pressure, and intracranial pressure.

The memories will be combined with sensors known to those of skill in the art as described herein [see, Examples],

and used to track the devices, to detect reactions or event, to detect and store the detected information, and to permit remote monitoring of patients or samples.

Also provided herein, are embodiments of matrices with memories in which the matrix is a sol-gel. The sol-gels, which are used to encapsulate biological molecules, such as molecules used as sensors, will include a memory device. For example, sol-gel biosensors are known. The improvement herein provides a means to store information regarding the sensed event or detect the event.

Also provided herein, are implantable biosensors that are coated with an angiogenic agent, such as an FGF, a VEGF, a PDGF, IL-8 and others. Upon implantation of the biosensor, agents will aid in directly blood flow to the device, thereby permitting the sensor to detect internal conditions more accurately and for an extended period of time.

The biosensors may also be combined with conducting polymers [see, Barisci et al. (1996) "Conducting Polymer Sensors", *TRIP* 4:307–311 to produce, for example, implantable drug delivery devices. These materials are polymers into which physiologically active substances, such as a hormone or enzyme, can be incorporated during or after copolymerization, and which will release the physiologically active substance upon application of a voltage. Combination of such polymers with a microprocessor with memory will permit timed release of the active molecule by the microprocessor. A second electrode **6510** coated with an electroactive polymer **6512** containing appropriate chemical dye is used to release the chemical dye for marking the bound antibodies and antigens.

a. Event-detecting Sensor

Another embodiment of the combinations herein uses a recording device that can detect the occurrence of a reaction or event or the status of any external parameter, such as pH or temperature, and record such occurrence or parameter in the memory, such embodiment is herein referred to as a sensor. Any of the above-described matrices with memories or memories may be modified to permit such detection. For example, the chip with the memory array with decoder, rectifier components and antenna, such as RF antenna, can be modified by addition of a photodetector and accompanying amplifier components as shown in FIG. **9**. The photodetector will be selected so that it is sensitive to the frequencies of expected photoemissions from reactions of interest. To maintain the chip's passive operation, the photodetector circuitry may use voltage supplied by the same RF signal that is used to write other data to memory, so that no detection of photoemission will occur unless RF or other power is applied to provide bias and drain voltage. If an active device is used, the power supplied by the battery can provide operational voltage to the photodetector circuitry, independent of any transmitted signal. The voltage supplied by the photodetector can be used in a number of different ways. For example:

1) The threshold voltage for writing to memory will exceed the voltage supplied by the RF signal, which will still contain the address information. In order to write, additional voltage must be provided by the photodetector so that the sum of the voltages exceeds the threshold. ($V_{RF}<V_T<V_{RF}+V_{PD}$). This permits the RF supplied voltage to go to the correct address, however, no writing will occur unless a photoemission has been detected by the detector. Therefore, there will be no record of exposure to a particular process step unless a sufficient reaction has occurred to generate the required photoemission. Since the address signal can still get to the memory array without the extra voltage, reading of recorded data can be achieved without any special circuitry. If the memory device is an active device, a similar mechanism can be used in which only the sum of the voltages is sufficient to record an occurrence.

2) The threshold voltage for writing to memory will be provided by the RF signal alone, and the RF signal will include address information. ($V_T<V_{RF}$). Unless voltage from the photodetector is supplied to a "gating" transistor, however, access to the memory array is prevented so that no writing occurs unless a photoemission is detected. (This embodiment is illustrated.) This will require a special provision for opening the gate during read operations to permit access to the memory array. Since the gating transistor will conduct a signal only in the event of photoemission, this embodiment will work equally well with passive and active memory devices.

3) The RF signal provides sufficient voltage to exceed the threshold voltage. ($V_T<V_{RF}$). Voltage from the photodetector is used to create a write potential difference at an additional address location which is carried in the RF signal. For example, if the RF signal is addressing column 3, row 3, column 32 could be connected only to the photodetector circuit's output so that, when a photoemission occurs, the write signal will create antifuses [or in the case of EEPROM, standard fuses] at addresses **3,3** and **32,3**. If no photoemission occurs, only address **3,3** will have an antifuse formed, providing a record of exposure of the matrix to a particular process step even without the occurrence of a detectable reaction. Special provisions, such as software within the host computer in combination with mask-programmed interconnections within the decode circuitry of the memory device, must be made to assure that more than one column in a single row of the array is polled during read operations so that both memory locations are read.

In addition to the above-described methods for recording the occurrence of photo-emitting reactions, the photodetector, while still integrated on the same substrate with the basic memory matrix for recording transmitted signals, can be connected to its own independent memory matrix. In this embodiment, the photodetector's memory matrix can be connected to separate transceiver circuitry with an antenna tuned to a different frequency from that of the basic memory. During the read operation, the memory device will be exposed to two different radio frequency signals, one for the basic memory, the other for the photodetection circuit memory. If only the photoemission information is required, only the corresponding frequency signal need be provided during the read operation.

Depending on the type of energy release that occurs during a reaction, other types of sensors may be used in addition to photodetectors or in place thereof. In addition changes in ion concentration may also be detected. Many such sensors will be capable of generating an electrical signal that can be used as described above for the photodetectors. These sensing devices may also be incorporated onto the substrate and electrically connected to the memory device, providing data points within the device's memory under the appropriate write conditions. For example, temperature sensing elements can be made from semiconductor liquid crystal and fluorescent crystals, and addition to conventional thermocouples created by placing two different metals in contact at the detection point. It is also possible to include radiation, pH and PCO_2 sensors in a similar manner, using materials that respond to the detected variables by generating a voltage potential that can be conducted to the memory device and recorded.

The reaction-detecting embodiment may be advantageously used in assays, such as the SPA, HTRF, FET, FRET and FP assays described below. In these assays, reaction, such as receptor binding, produces a detectable signal, such as light, in the matrix. If a matrix with memory with a photodetection circuit is used, occurrence of the binding reaction will be recorded in memory.

b. Devices for Drug Delivery and Sensors for Detecting Changes in Internal Conditions in the Body

Memories may also be combined with biocompatible supports and polymers that are used internally in the bodies of animals, such as drug delivery devices [see, e.g., U.S. Pat. Nos. 5,447,533, 5,443,953, 5,383,873, 5,366,733, 5,324,324, 5,236,355, 5,114,719, 4,786,277, 4,779,806, 4,705,503, 4,702,732, 4,657,543, 4,542,025, 4,530,840, 4,450,150 and 4,351,337] or other biocompatible support [see, U.S. Pat. No. 5,217,743 and U.S. Pat. No. 4,973,493, which provide methods for enhancing the biocompatibility of matrix polymers). Such biocompatible polymers include matrices of poly(ethylene-co-vinyl acetate) and matrices of a polyanhydride copolymer of a stearic acid dimer and sebacic acid [see, e.g., Sherwood et al. (1992) *Bio/Technology* 10:1446–1449].

The biocompatible drug delivery device in combination with the memory is introduced into the body. The device, generally by virtue of combination with a biosensor or other sensor, also monitors pH, temperature, electrolyte concentrations and other such physiological parameters and in response to preprogrammed changes, directs the drug delivery device to release or not release drugs or can be queried, whereby the change is detected and drug delivered or administered.

Alternatively, the device provided in combination with a biocompatible support and biosensor, such that the information determined by the biosensor can be stored in the device memory. The combination of device and biosensor is introduced into the body and is used to monitor internal conditions, such as glucose level, which level is written to memory. The internal condition, such as glucose level, electrolytes, particularly potassium, pH, hormone levels, and other such level, can then be determined by querying the device.

In one embodiment, the device, such as one containing a memory that is read to and written using RF, linked to a biosensor [see, e.g., U.S. Pat. No. 5,384,028 which provides a biosensor with a data memory that stores data] that can detect a change in an internal condition, such as glucose or electrolyte, and store or report that change via RF to the linked matrix with memory, which records such change as a data point in the memory, which can then be queried. The animal is then scanned with RF and the presence of the data point is indicative of a change. Thus, instead of sampling the body fluid, the memory with matrix with linked biosensor is introduced into a site in the body, and can be queried externally. For example, the sensor can be embedded under the skin and scanned periodically, or the scanner is worn on the body, such as on the wrist, and the matrix with memory either periodically, intermittently, or continuously sends signals; the scanner is linked to an infusion device and automatically, when triggered triggers infusion or alters infusion rate.

A well-known problem that can rapidly render a sensing implant ineffective is the body's natural response to "wall-off" encapsulate the implant such that the concentration of any analyte in this poorly vascularized tissue surrounding the implant does not properly reflect the analyte concentration in tissue as a whole. A method for solving this problem is provided herein. Any implantable biosensor may be modified by including an angiogenic material in the matrix that surrounds the implant. The angiogenic material will promote the growth of new vascularization into the tissue immediately surrounding the implant and thus improve transport of analyte to the sensor implant. Examples of angiogenic factors, include, but are not limited to basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), heparin, prostaglandin E1, and interleukins, such as IL-1a and IL-8, among others.

Also, the implant may be coated with collagen and/or heparin to which the angiogenic factor, such as bFGF, is absorbed, thereby presenting the factor in a configuration that mimics its in vivo presentation.

In addition, these factors may be provided in a time release format, such as by containing the angiogenic factor within a collagen gel in conjunction with, for example, an aluminum sucrose octasulfate suspension.

4. Reading and Writing to Memory

a. Embodiments Using a Proximate Memory, Such as a Non-volatile Memory Device

The operation of programming the memory to record the process steps to which the linked or adjacent matrix particle or support and linked or proximate molecule or biological particle is exposed involves placing the memory device reasonably close. A distance on the order of about 1 inch (25.4 mm) to RF transmitter **50** is presently contemplated, but longer distances should be possible and shorter distances are also contemplated. Suitable distances can be determined empirically. The RF transmitter **50** emits a carrier wave modulated by a signal generated by host computer **70** using conventional RF technology. The carrier wave itself can provide the power to the generate the programming voltage and the operating voltage for the various devices via the rectifier, while the modulation signal provides the address instructions.

As stated previously, since the memory only has to be "tagged" to record the exposure of the proximate or linked molecule or biological particle to a given process, the address signal only has to carry information to turn on a single memory location, while the host computer **70** stores into memory **68** the information linking the process information with the single memory location that was "tagged" to record exposure to the process step. Referring to FIG. **1**, in which chemical building blocks A, C, and E are added to a molecule linked to a matrix with memory, and to FIG. **6**, an illustrative example of how information is written onto a particle is provided in Table 1.

TABLE 1

PROCESS STEP	X-REGISTER ADDRESS	Y-REGISTER ADDRESS
A	1	8
C	2	4
E	3	2

For the step in which A is added, the address signal would increment the x-register **124** one location and increment the

y-register **126** eight locations, and then apply the programming voltage. The activation of this switch is indicated by an "A" at the selected address, although the actual value stored will be a binary "1", indicating ON. (As described, for example, in U.S. Pat. No. 4,424,579; the manner in which the programming voltage is applied depends on whether the decoders have depletion or enhancement transistors.) The host computer **70** would write into its memory **148** that for process A, the x-,y- address is 1,8. Upon removal of the RF signal after recording process A, the voltage is removed and the registers would reset to 0. For the step in which C is added, the address signal would increment the x-register **124** two locations and the y-register **126** four locations, then apply the programming voltage, as indicated by the letter "C". The host computer **70** would similarly record in memory that an indication of exposure to process C would be found at x-,y- address 2,4. Again, upon removal of the RF signal, the registers reset to 0 so that when the matrix particle's memory is again exposed to RF following addition of block E, the registers increment 3 and 2 locations, respectively, and the programming voltage is applied to turn on the switch, indicated by "E". Desirably all processing steps are automated.

After processing is completed, to read the information that has been recorded in the memory of the data storage unit, the host computer **70** will inquire into the identity of the particle by generating a command signal to the registers to select the appropriate address locations to determine whether the switch is on or off. If the switch is on, i.e., a voltage drop occurs at that point, the computer will create a record that the particle received a particular process step. Alternatively, the host computer can generate an inquiry signal to sequentially look at all memory locations to determine which switches have been turned on, recording all locations at which voltage drops occurred. The computer will then compare the "on" locations to the process steps stored in its memory to identify the steps through which the subject particle was processed.

If desired, individual particles can be identified by reserving certain memory locations for identification only, for example, the first two rows of the x-register. In this case, particles will be passed separately through the RF signal while the x-register is incremented to turn on switches at address locations 0,0, 1,0, 2,0, etc. With individual identification, the host computer **70** can first generate a signal to query a matrix particle memory to determine its identity, then write the information with regard to the process performed, saving the process and particle information in the host computer memory **68**.

Ideally, the tagging of particles which are exposed to a particular process would be performed in the process vessel containing all of the particles. The presence, however, of a large number of particles may result in interference or result in an inability to generate a sufficiently high voltage for programming all of the particles simultaneously. This might be remedied by providing an exposure of prolonged duration, e.g., several minutes, while stirring the vessel contents to provide the greatest opportunity for all particles to receive exposure to the RF signal. On the other hand, since each particle will need to be read individually, a mechanism for separating the particles may be used in write and read operations. Also, in instances in which each particle will have a different molecule attached, each particle memory must be addressed separately.

b. Embodiments Using OMDs

When precoded OMDs are used, each OMD (or group thereof) has a unique identifier is optically scanned and entered into a remote memory. Thereafter, after each synthesis, processing or assaying step, information regarding such for each device identified by its encoded symbology is entered into a remote memory. Upon completion of the synthesis, processing, assay or other protocol, each device can be scanned and identified. Reference to the information stored in the remote memory provides information regarding the linked molecules or biological particles or the assay or other information. When read/write OMDs are used, identifying symbology is encoded on the device and the decrypting information is stored in a remote memory.

D. The combinations and preparation thereof

Combinations of a memory, such as an optical memory, a magnetic memory or a miniature recording device, that contains or is a data storage unit linked to or in proximity with matrices or supports used in chemical and biotechnical applications, such as combinatorial chemistry, peptide synthesis, nucleic acid synthesis, nucleic acid amplification methods, organic template chemistry, nucleic acid sequencing, screening for drugs, particularly high throughput screening, phage display screening, cell sorting, drug delivery, tracking of biological particles and other such methods, are provided. These combinations of matrix material with data storage unit [or recording device including the unit] are herein referred to as matrices with memories. These combinations have a multiplicity of applications, including combinatorial chemistry, isolation and purification of target macromolecules, capture and detection of macromolecules for analytical purposes, high throughput screening protocols, selective removal of contaminants, enzymatic catalysis, drug delivery, chemical modification, scintillation proximity assays, FET, FRET and HTRF assays, immunoassays, receptor binding assays, drug screening assays, information collection and management and other uses. These combinations are particularly advantageous for use in multianalyte analyses. These combinations may also be advantageously used in assays in which a electromagnetic signal is generated by the reactants or products in the assay. These combinations may be used in conjunction with or may include a sensor element, such as an element that measures a solution parameter, such as pH. Change in such parameter, which is recorded in the memory will indicate a reaction event of interest, such as induction of activity of a receptor or ion channel, has occurred. The combination of matrix with memory is also advantageously used in multiplex protocols, such as those in which a molecule is synthesized on the matrix, its identity recorded in the matrix, the resulting combination is used in an assay or in a hybridization reaction. Occurrence of the reaction can be detected externally, such as in a scintillation counter, or can be detected by a sensor that writes to the memory in the matrix. Thus, combinations of matrix materials, memories, and linked or proximate molecules and biological materials and assays using such combinations are provided.

The combinations contain (i) a miniature recording device that contains one or more programmable data storage devices [memories] that can be remotely read and in preferred embodiments also remotely programmed; and (ii) a matrix as described above, such as a particulate support used in chemical syntheses. The remote programming and reading is preferably effected using electromagnetic radiation, particularly radio frequency or radar, microwave, X-rays. Depending upon the application the combinations will include additional elements, such as scintillants, photodetectors, pH sensors and/or other sensors, and other such elements.

1. Preparation of Matrix-memory Combinations

In preferred embodiments, the recording device is cast in a selected matrix material during manufacture.

Alternatively, the devices can be physically inserted into the matrix material, the deformable gel-like materials, or can be placed on the matrix material and attached by a connector, such as a plastic or wax or other such material. Alternatively, the device or device(s) may be included in an inert container in proximity to or in contact with matrix material.

2. Non-linked matrix-memory Combinations

The recording device with memory can be placed onto the inner or outer surface of a vessel, such as a microtiter plate or vial or tube in which the reaction steps are conducted, fractions collected or samples stored. Alternatively, the device can be incorporated into the vessel material, such into the a wall of each microtiter well or vial or tube in which the reaction is conducted. As long as the molecules or biological particles remain associated with the well, tube or vial, such as a vial used to collect HPLC fractions, or a vial containing a patient sample, their identity can be tracked. The memory will be a programmable electronic memory or a bar code. These memories can also be associated with reagent containers.

A bar code reader, transponder reader or other such device can be used to enter the desired information building block name by reading the reagent container. Such information will be entered in to matrix memory or remote computer memory. Software for doing so can be integrated into the systems used, such as the bar code reader described herein or the transponders and encoders described herein.

Also of interest herein are the multiwell "chips" [such as those available from Orchid Biocomputer, Inc. Princeton, N.J., see, e.g., U.S. Pat. Nos. 5,047,371, 4,952,531, 5,043, 222, 5,277,724, 5,256,469 and Prabhu et al. (1992) *Proc. SPIE-Int. Soc. Opt. Eng.* 1847 NUMBER: Proceedings of the 1992 International Symposium on Microelectronics, pp.601–6, that are silicone based chips that contain 10,000 microscopic wells connected by hair-thin glass tubes to tiny reservoirs containing reagents for synthesis of compounds in each well. Each well can be marked with a code and the code associated with the identity of the synthesized compound in each well. Ultimately, a readable or read/write memory may be incorporated into each well, thus permitting rapid and ready identification of the contents of each well.

In a particularly preferred embodiment, one or more recording devices with memory and matrix particles are sealed in a porous non-reactive material, such as polypropylene or TEFLON net, with a pore size smaller than the particle size of the matrix and the device. Typically one device per about 1 to 50 mg, preferably 5 to 30, more preferably 5 to 20 mg of matrix material, or in some embodiments up to gram, generally 50 to 250 mg, preferably 150 mg to about 200 mg, and one device is sealed in a porous vessel a microvessel [MICROKAN™]. The amount of matrix material is a function of the size of the device and the application in which the resulting matrix with memory is used, and, if necessary can be empirically determined. Generally, smaller sizes are desired, and the amount of material will depend upon the size of the selected recording device.

The resulting microvessels are then encoded, reactions, such as synthetic reactions, performed, and read, and if desired used in desired assays or other methods.

3. Combinations of Memories With Sensors and Matrices With Memories and Sensors

As discussed above, below, and exemplified below, combinations of sensors with memories and sensors with matrices with memories are provided. Also provided are sol-gels with memories, coated sensors and other embodiments.

4. Preparation of Matrix-memory-molecule or Biological Particle Combinations

In certain embodiments, combinations of matrices with memories and biological particle combinations are prepared. For example, libraries [e.g., bacteria or bacteriophage, or other virus particles or other particles that contain genetic coding information or other information] can be prepared on the matrices with memories, and stored as such for future use or antibodies can be linked to the matrices with memories and stored for future use.

5. Combinations For Use in Proximity Assays

In other embodiments the memory or recording device is coated or encapsulated in a medium, such as a gel, that contains one or more fluophors or one or more scintillants, such as 2,5-diphenyloxazole [PPO] and/or 1,4-bis-[5-phenyl-(oxazolyl)]benzene [POPOP] or FlexiScint [a gel with scintillant available from Packard, Meriden, Conn.] or yttrium silicates. Any fluophore or scintillant or scintillation cocktail known to those of skill in the art may be used. The gel coated or encased device is then coated with a matrix suitable, such as glass or polystyrene, for the intended application or applications. The resulting device is particularly suitable for use as a matrix for synthesis of libraries and subsequent use thereof in scintillation proximity assays.

Similar combinations in non-radioactive energy transfer proximity assays, such as HTRF, FP, FET and FRET assays, which are described below. These luminescence assays are based on energy transfer between a donor luminescent abel, such as a rare earth metal cryptate [e.g., Eu trisbipyridine diamine (EuTBP) or Tb tribipyridine diamine (TbTBP)] and an acceptor luminescent label, such as, when the donor is EuTBP, allopycocyanin (APC), allophycocyanin B, phycocyanin C or phycocyanin R, and when the donor is TbTBP, a rhodamine, thiomine, phycocyanin R, phycoerythrocyanin, phycoerythrin C, phycoerythrin B or phycoerythrin R. Instead of including a scintillant in the combination, a suitable fluorescent material, such as allopycocyanin (APC), allophycocyanin B, phycocyanin C, phycocyanin R; rhodamine, thiomine, phycocyanin R, phycoerythrocyanin, phycoerythrin C, phycoerythrin B or phycoerythrin R is included. Alternatively, a fluorescent material, such a europium cryptate is incorporated in the combination.

6. 2-D Bar Codes, Other Symbologies and Application Thereof

Any application and combination described herein in which a recording device in proximity with a matrix may include a code or symbology in place of or in addition to the recording device. The information associated with the code is stored in a remote recording device, such as a computer. Thus, by electro-optically scanning the symbol on the combination and generating a corresponding signal, it is possible in an associated computer whose memory has digitally stored therein the full range of codes, to compare the signal derived from the scanned symbol with the stored information. When a match is found, the identity of the item and associated information, such as the identity of the linked molecule or biological particle or the synthetic steps or assay protocol, can be retrieved.

The symbology can be engraved on any matrix used as a solid support for chemical syntheses, reactions, assays and

other uses set forth herein, for identification and tracking of the linked or proximate biological particles and molecules. Particularly preferred is the two-dimensional bar code and system used therewith for reading and writing the codes on matrix materials.

7. Other Variations and Embodiments

The combination of matrix particle with memory may be further linked, such as by welding using a laser or heat, to an inert carrier or other support, such as a TEFLON strip. This strip, which can be of any convenient size, such as 1 to 10 mm by about 10 to 100 μM will render the combination easy to use and manipulate. For example, these memories with strips can be introduced into 10 cm culture dishes and used in assays, such as immunoassays, or they can be used to introduce bacteria or phage into cultures and used in selection assays. The strip may be encoded or impregnated with a bar code to further provide identifying information.

Microplates containing a recording device in one or a plurality of wells are provided. The plates may further contain embedded scintillant or a coating of scintillant [such as FlashPlate™, available from DuPont NEN™, Cytostar-T plates from Amersham International plc, U.K., and plates available from Packard, Meriden, CT] FLASHPLATE™ is a 96 well microplate that is precoated with plastic scintillant for detection of β-emitting isotopes, such as ^{125}I, ^{3}H, ^{35}S, ^{14}C and ^{33}P. A molecule is immobilized or synthesized in each well of the plate, each memory is programmed with the identify of each molecule in each well. The immobilized molecule on the surface of the well captures a radiolabeled ligand in solution results in detection of the bound radioactivity. These plates can be used for a variety of radioimmmunoassays [RIAs], radioreceptor assays [RRAs], nucleic acid/protein binding assays, enzymatic assays and cell-based assays, in which cells are grown on the plates.

Another embodiment is depicted in FIG. **19**. The reactive sites, such as amines, on a support matrix [**1** in the FIGURE] in combination with a memory [a MICROKAN™, a MICROTUBE™, a MACROBEAD™, a MICROCUBE™ or other matrix with memory combination] are differentiated by reacting them with a selected reaction of Fmoc-glycine and Boc-glycine, thereby producing a differentiated support [**2**]. The Boc groups on **2** are then deprotected with a suitable agent such as TFA, to produce **3**. The resulting fee amine groups are coupled with a fluophore [or mixture A and B], to produce a fluorescent support **4**, which can be used in subsequent syntheses or for linkage of desired molecules or biological particles, and then used in fluorescence assays and SPAs.

E. The recording and reading and systems

Systems for recording and reading information are provided. The systems include a host computer or decoder/encoder instrument, a transmitter, a receiver and the data storage device. The systems also can include a funnel-like device and other sorting devices for use in separating and/or tagging single memory devices. In practice, an EM signal or other signal is transmitted to the data storage device. The antenna or other receiver means in the device detects the signal and transmits it to the memory, whereby the data are written to the memory and stored in a memory location. As provided herein, methods for addressing individual memories in a batch are provided, thereby eliminating the need for sorting.

Mixtures of the matrix with memory-linked molecules or biological particles may be exposed to the EM signal, or each matrix with memory [either before, after or during linkage of the biological particles or molecules] may be individually exposed, using a device, such as that depicted herein, to the EM signal. Each matrix with memory, as discussed below, will be linked to a plurality of molecules or biological particles, which may be identical or substantially identical or a mixture of molecules or biological particles depending, upon the application and protocol in which the matrix with memory and linked [or proximate] molecules or biological particles is used. The memory can be programmed with data regarding such parameters.

The location of the data, which when read and transmitted to the host computer or decoder/encoder instrument, corresponds to identifying information about linked or proximate molecules or biological particles. The host computer or decoder/encoder instrument can either identify the location of the data for interpretation by a human or another computer or the host computer or the decoder/encoder can be programmed with a key to interpret or decode the data and thereby identify the linked molecule or biological particle.

As discussed above, the presently preferred system for use is the IPTT-100 transponder and DAS-5001 CONSOLE™ [Bio Medic Data Systems, Inc., Maywood, N.J.; see, e.g., U.S. Pat. Nos. 5,422,636, 5,420,579, 5,262,772, 5,252,962 and 5,250,962, 5,252,962 and 5,262,772].

These systems may be automated or may be manual.

F. Manual system

The manual system includes a memories with matrices, a device for reading and writing thereto and software therefor. The presently preferred manual system in which the matrices with memories have electromagnetically programmable memories, includes a transponder, particularly the BMDS transponder described below or an IDTAG transponder or the monolithic chip provided herere, or any suitable read or read/write memory device and uses the corresponding reading and writing device, which has been reconfigured and repackaged, such as in FIG. **17**, described in the EXAMPLES. An example of the operation of the system of FIG. **17** is illustrated in FIG. **18** and described in EXAMPLE 4. Briefly, the user manually places a microvessel **180** with memory within the recessed area **176** so that the interrogation signal **185** provides a response to the controllers indicating the presence on the microvessel, and information is read from or written to the transponder.,

This will include microvessels, such as MICROKANS™ or MICROTUBES™, MICROBEADS™, MICROBALS™ read/writer hardware [such as that available from BMDS or IDTAG™] connected to a PC and software running on the PC that performs a user interface and system control function. The software is designed to facilitate the a number of aspects of synthetic combinatorial chemistry libraries, including: organization, planning and design, synthesis compound formula determination, molecular weight computation, reporting of plans, status and results.

In particular, for each chemical library, the software creates a data base file. This file contains all of the information pertinent to the library, including chemical building blocks to be used, the design of the library in terms of steps and splits, and what synthesis has been performed. This file oriented approach allows many different chemical library projects to be conducted simultaneously. The software allows the user to specify what chemical building blocks are to be used and their molecular weights. The user specifies the number of steps, the number of "splits" at each step, and what chemical building blocks are to be used at each split. The user may also enter the name of the pharmacophore and its molecular weight. Additionally, the user may specify

graphical chemical diagrams for the building blocks and the pharmacophore. This information is useful in displaying resulting compounds. The software records all of the above "design" information. It computes and displays the size of the library. It may also predict the range of molecular weights of the resulting compounds.

For example, the user specifies that there will be eight chemical building blocks. Their names are entered, and the user enters a unique letter codes for each: A, B, C, D, E, F, G and H. The user specifies that there will be three steps. Step one will have four splits, appending the A, B, C and D building blocks. Step two will also have four splits, adding the B, D, E and H building blocks. Step three will have six splits, adding the B, C, D, E, F and G building blocks. The software computes that the library will contain 96 (4×6×5=96) unique compounds. With the planning and design completed, the software helps the user perform the synthesis steps. This is done in concert with the reader/writer hardware [transceiver or a scanner, such as the BMDS—DAS 5003] or a similar device available form IDTAG Ltd [Bracknell, Berks RG12 3XQ, UK] and devices, such as the MICROKAN™ or MICROTUBE™ microvessel with memory devices. Before the synthesis begins, the microvessels are filled with polymer resin. The microvessel devices are, one at a time placed upon the scanner. The device and software reads the contents of the data encoded in the recording device, transponder, such as the BMDS tag or the IDTAG™ tag, contained in each microvessel. The software, chooses which building block shall be added to the compound contained in each microvessel. It directs the transceiver to write encoded data to the transponder, indicating which building block this is. The software displays a message which directs the user to place the microvessel in the appropriate reaction vessel so that the chosen building block will be added. This process is repeated a plurality of times with each microvessel and for each synthetic step the planned steps of the library.

The software then uses the scanner to read a tag and receive its encoded information. Using the user-entered compound names stored in the library's data base, the software translates the encoded information into the names of the chemical building blocks. The software can also display compounds graphically, using the graphical information specified by the user. The software calculates the molecular weight of compounds from the data provided for the pharmacophore and building blocks.

The software facilitates the recording of progress through the above process. The software generates displays and reports which illustrate this and all of the above planning, design, compound data, and graphical representations of compounds. An example of the software [to be commercialized under the name Synthesis Manager] and use thereof is described in the Examples, below. Once armed with the instant disclosure, other such software can be developed by one skilled in that art.

Briefly, in the first step in building a library, the individual building blocks [i.e., the monomers, nucleotides or amino acids or other small molecules] and the steps in which they will be used are defined. The software then automatically creates a data base record for each compound to be synthesized. Pre-reaction procedures, reaction conditions, and work up procedures are stored for each step.

When the synthesis begins, the step "Perform Synthesis" is selected. The software plays back to the user the procedure, and then reads each of the memories in each microreactor and sorts them for the next reaction step. When the sorting is complete, the reaction condition information and work-up procedure are also displayed to the user.

When the chemical synthesis is complete, compounds are cleaved from the microreactors and archived. The software provides archival capability for either individual vials or a 96-well format or will be adapted for other formats. Specific columns, rows, or individual well scan be protected to accommodate the need for standards and controls in virtually any screening format.

The software provides several utilities that permit one tag to be read at any time, display the corresponding building block names and structures, and the current synthesis status of that compound. It is also possible to search for a specific compound or compounds that contain certain building blocks. For compounds that have already been archived, the archive location [i.e., microplate group name, number, and well] will be displayed.

G. Tools and Applications using matrices with memories

1. Tools

The matrix with memory and associated system as described herein is the basic tool that can be used in a multitude of applications, including any reaction that incorporates a functionally specific (i.e. in the reaction) interaction, such as receptor binding. This tool is then combined with existing technologies or can be modified to produce additional tools.

For example, the matrix with memory combination, can be designed as a single analyte test or as a multianalyte test and also as a multiplexed assay that is readily automated. The ability to add one or a mixture of matrices with memories, each with linked or proximate molecule or biological particle to a sample, provides that ability to simultaneously determine multiple analytes and to also avoid multiple pipetting steps. The ability to add a matrix with memory and linked molecules or particles with additional reagents, such as scintillants, provides the ability to multiplex assays.

As discussed herein, in one preferred embodiment the matrices are particulate and include adsorbed, absorbed, or otherwise linked or proximate, molecules, such as peptides or oligonucleotides, or biological particles, such as cells. Assays using such particulate memories with matrices may be conduced "on bead" or "off bead". On bead assays are suitable for multianalyte assays in which mixtures of matrices with linked molecules are used and screened against a labeled known. Off bead assays may also be performed; in these instances the identity of the linked molecule or biological particle must be known prior to cleavage or the molecule or biological particle must be in some manner associated with the memory.

In other embodiments the matrices with memories use matrices that are continuous, such as microplates, and include a plurality of memories, preferably one memory/well. Of particular interest herein are matrices, such as Flash Plates™ [NEN, Dupont], that are coated or impregnated with scintillant or fluophore or other luminescent moiety or combination thereof, modified by including a memory in each well. The resulting matrix with memory is herein referred to as a luminescing matrix with memory. Other formats of interest that can be modified by including a memory in a matrix include the Multiscreen Assay System [Millipore] and gel permeation technology. Again it is noted that the memories may be replaced with or supplemented with engraved code, preferably at the base of each well [outer surface preferred] that is either precoded or added prior to or during use. The memory, in these instances, is then remote from the matrix.

Preferred plates are those that contain a microplate type frame and removable wells or strips. Each well or strip can contain a memory and/or can be engraved with a code.

2. Scintillation Proximity Assays (SPAs) and Scintillant-containing Matrices with Memories

Scintillation proximity assays are well known in the art [see, e.g., U.S. Pat. No. 4,271,139; U.S. Pat. No. 4,382,074; U.S. Pat. No. 4,687,636; U.S. Pat. No. 4,568,649; U.S. Pat. No. 4,388,296; U.S. Pat. No. 5,246,869; International PCT Application No. WO 94/26413; International PCT Application No. WO 90/03844; European Patent Application No. 0 556 005 A1; European Patent Application No. 0 301 769 A1; Hart et al. (1979) *Molec. Immunol.* 16:265–267; Udenfriend et al. (1985) *Proc. Natl. Acad. Sci. U.S.A.* 82:8672–8676; Nelson et al. (1987) *Analyt. Biochem* 165:287–293; Heath, et al. (1991) *Methodol. Surv. Biochem. Anal.* 21:193–194; Mattingly et al. (1995) *J. Memb. Sci.* 98:275–280; Pernelle (1993) *Biochemistry* 32:11682–116878; Bosworth et al. (1989) *Nature* 341:167–168; and Hart et al. (1989) *Nature* 341:265]. Beads [particles] and other formats, such as plates and membranes have been developed.

SPA assays refer to homogeneous assays in which quantifiable light energy produced and is related to the amount of radioactively labelled products in the medium. The light is produced by a scintillant that is incorporated or impregnated or otherwise a part of a support matrix. The support matrix is coated with a receptor, ligand or other capture molecule that can specifically bind to a radiolabeled analyte, such as a ligand.

a. Matrices

Typically, SPA uses fluomicrospheres, such as diphenyloxazole-latex, polyacrylamide-containing a fluophore, and polyvinyltoluene [PVT] plastic scintillator beads, and they are prepared for use by adsorbing compounds into the matrix. Also fluomicrospheres based on organic phosphors have been developed. Microplates made from scintillation plastic, such as PVT, have also been used [see, e.g., International PCT Application No. WO 90/03844]. Numerous other formats are presently available, and any format may be modified for use herein by including one or more recording devices.

Typically the fluomicrospheres or plates are coated with acceptor molecules, such as receptors or antibodies to which ligand binds selectively and reversibly. Initially these assays were performed using glass beads containing fluors and functionalized with recognition groups for binding specific ligands [or receptors], such as organic molecules, proteins, antibodies, and other such molecules. Generally the support bodies used in these assays are prepared by forming a porous amorphous microscopic particle, referred to as a bead [see, e.g., European Patent Application No.0 154,734 and International PCT Application No. WO 91/08489]. The bead is formed from a matrix material such as acrylamide, acrylic acid, polymers of styrene, agar, agarose, polystyrene, and other such materials, such as those set forth above. Cyanogen bromide has been incorporated into the bead into to provide moieties for linkage of capture molecules or biological particles to the surface. Scintillant material is impregnated or incorporated into the bead by precipitation or other suitable method. Alternatively, the matrices are formed from scintillating material [see, e.g., International PCT Application No. WO 91/08489, which is based on U.S. application Ser. No. 07/444,297; see, also U.S. Pat. No. 5,198,670], such as yttrium silicates and other glasses, which when activated or doped respond as scintillators. Dopants include Mn, Cu, Pb, Sn, Au, Ag, Sm, and Ce. These materials can be formed into particles or into continuous matrices. For purposes herein, the are used to coat, encase or otherwise be in contact with one or a plurality of recording devices.

Assays are conducted in normal assay buffers and requires the use of a ligand labelled with an isotope, such as ^{3}H and ^{125}I, that emits low-energy radiation that is readily dissipated easily an aqueous medium. Because ^{3}H β particles and ^{125}I Auger electrons have average energies of 6 and 35 keV, respectively, their energies are absorbed by the aqueous solutions within very small distances (~4 μm for ^{3}H β particles and 35 Arm for ^{125}I Auger electrons). Thus, in a typical reaction of 0.1 ml to 0.4 ml the majority of unbound labelled ligands will be too far from the fluoromicrosphere to activate the fluor. Bound ligands, however, will be in sufficiently close proximity to the fluomicrospheres to allow the emitted energy to activate the fluor and produce light. As a result bound ligands produce light, but free ligands do not. Thus, assay beads emit light when they are exposed to the radioactive energy from the label bound to the beads through the antigen-antibody linkage, but the unreacted radioactive species in solution is too far from the bead to elicit light. The light from the beads will be measured in a liquid scintillation counter and will be a measure of the bound label.

Matrices with memories for use in scintillation proximity assays [SPA] are prepared by associating a memory [or engraved or imprinted code or symbology) with a matrix that includes a scintillant. In the most simple embodiment, matrix particles with scintillant [fluomicrospheres] are purchased from Amersham, Packard, NE Technologies [(formerly Nuclear Enterprises, Inc.) San Carlos, Calif.] or other such source and are associated with a memory, such as by including one or more of such beads in a MICROKAN™ microvessel with a recording device. Typically, such beads as purchased are derivatized and coated with selected moieties, such as streptavidin, protein A, biotin, wheat germ agglutinin [WGA], and polylysine. Also available are inorganic fluomicrospheres based on cerium-doped yttrium silicate or polyvinyltoluene (PVT). These contain scintillant and may be coated and derivatized.

Alternatively, small particles of PVT impregnated with scintillant are used to coat recording devices, such as the IPTT-100 devices [Bio Medic Data Systems, Inc., Maywood, N.J.; see, also U.S. Pat. Nos. 5,422,636, 5,420, 579, 5,262,772, 5,252,962, 5,250,962, 5,074,318, and RE 34,936] that have been coated with a protective material, such as polystyrene, TEFLON, a ceramic or anything that does not interfere with the reading and writing EM frequency(ies). Such PVT particles may be manufactured or purchased from commercial sources such as NE TECHNOLOGY, INC. [e.g., catalog #191A, 1–10 μm particles]. These particles are mixed with agarose or acrylamide, styrene, vinyl or other suitable monomer that will polymerize or gel to form a layer of this material, which is coated on polystyrene or other protective layer on the recording device. The thickness of the layers may be empirically determined, but they must be sufficiently thin for the scintillant to detect proximate radiolabels. To make the resulting particles resistant to chemical reaction they may be coated with polymers such as polyvinyltoluene or polystyrene, which can then be further derivatized for linkage and/or synthesis of molecules and biological particles. The resulting beads are herein called luminescing matrices with memories, and when used in SPA formats are herein referred to as scintillating matrices with memories.

The scintillating matrices with memories beads can be formed by manufacturing a bead containing a recording device and including scintillant, such as 2,5-diphenyloxazole [PPO] and/or 1,4-bis-[5-phenyl-(oxazolyl)]benzene [POPOP] as a coating. These particles or beads are then coated with derivatized polyvinyl benzene or other suitable matrix on which organic synthesis, protein synthesis or other synthesis can be performed or to which organic molecules, proteins, nucleic acids, biological particles or other such materials can be attached. Attachment may be effected using any of the methods known to those of skill in the art, including methods described herein, and include covalent, non-covalent, direct and indirect linkages.

Alternatively or additionally, each bead may be engraved with a code. Preferably the beads are of such geometry that they can be readily oriented for reading.

Molecules, such as ligands or receptors or biological particles are covalently coupled thereto, and their identity is recorded in the memory. Alternatively, molecules, such as small organics, peptides and oligonucleoties, are synthesized on the beads as described herein so that history of synthesis and/or identity of the linked molecule is recorded in the memory. The resulting matrices with memory particles with linked molecules or biological particles may be used in any application in which SPA is appropriate. Such applications, include, but are not limited to: radioimmunoassays, receptor binding assays, enzyme assays and cell biochemistry assays.

For use herein, the beads, plates and membranes are either combined with a recording device or a plurality of devices, or the materials used in preparing the beads, plates or membranes is used to coat, encase or contact a recording device and/or engraved with a code. Thus, microvessels [MICROKANS™] containing SPA beads coated with a molecule or biological particle of interest; microplates impregnated with or coated with scintillant, and recording devices otherwise coated with, impregnated with or contacted with scintillant are provided.

To increase photon yield and remove the possibility of loss of fluor, derivatized fluomicrospheres based on yttrium silicate, that is doped selectively with rare earth elements to facilitate production of light with optimum emission characteristics for photomultipliers and electronic circuitry have been developed [see, e.g., European Patent Application No. 0 378 059 B1; U.S. Pat. No. 5,246,869]. In practice, solid scintillant fibers, such as cerium-loaded glass or based on rare earths, such as yttrium silicate, are formed into a matrix. The glasses may also include activators, such as terbium, europium or lithium. Alternatively, the fiber matrix may be made from a scintillant loaded polymer, such as polyvinyltoluene. Molecules and biological particles can be adsorbed to the resulting matrix.

For use herein, these fibers may be combined in a microvessel with a recording device [i.e., to form a MICROKAN™]. Alternatively, the fibers are used to coat a recording device or to coat or form a microplate containing recording devices in each well. The resulting combinations are used as supports for synthesis of molecules or for linking biological particles or molecules. The identity and/or location and/or other information about the particles is encoded in the memory and the resulting combinations are used in scintillation proximity assays.

Scintillation plates [e.g., FlashPlates™, NEN Dupont, and other such plates] and membranes have also been developed [see, Mattingly et al. (1995) *J. Memb. Sci.* 98:275–280] that may be modified by including a memory for use as described herein. The membranes, which can contain polysulfone resin M.W. 752 kD, polyvinylpyrrolidone MW 40 kDA, sulfonated polysulfone, fluor, such as p-bis-o-methylstyrylbenzene, POP and POPOP, may be prepared as described by Mattingly, but used to coat, encase or contact a recording device. Thus, instead of applying the polymer solution to a glass plate the polymer solution is applied to the recording device, which, if need is pre-coated with a protective coating, such as a glass, TEFLON or other such coating.

Further, as shown in the Examples, the recording device may be coated with glass, etched and the coated with a layer of scintillant. The scintillant may be formed from a polymer, such as polyacrylamide, gelatin, agarose or other suitable material, containing fluophors, a scintillation cocktail, FlexiScint [Packard Instrument Co., Inc., Downers Grove, Ill.] NE Technology beads [see, e.g., U.S. Pat. No. 4,588,698 for a description of the preparation of such mixtures]. Alternatively, microplates that contain recording devices in one or more wells may be coated with or impregnated with a scintillant or microplates containing scintillant plastic may be manufactured with recording devices in each well. If necessary, the resulting bead, particle or continuous matrix, such as a microplate, may be coated with a thin layer polystyrene, TEFLON or other suitable material. In all embodiments it is critical that the scintillant be in sufficient proximity to the linked molecule or biological particle to detect proximate radioactivity upon interaction of labeled molecules or labeled particles with the linked molecule or biological particle.

The resulting scintillating matrices may be used in any application for which scintillation proximity assays are used. These include, ligand identification, single assays, multianalyte assays, including multi-ligand and multi-receptor assays, radioimmunoassays [RIAs], enzyme assays, and cell bio-chemistry assays [see, e.g., International PCT Application No. WO 93/19175, U.S. Pat. No. 5,430,150, Whitford et al. (1991) *Phytochemical Analysis* 2:134–136; Fenwick et al. (1994) *Anal. Proc. Including Anal. Commun.* 31:103–106; Skinner et al. (1994) *Anal. Biochem.* 223:259–265; Matsumura et al. (1992) *Life Sciences* 51:1603–1611; Cook et al. (1991) *Structure and Function of the Aspartic Proteinases*, Dunn, ed., Penum Press, NY, pp. 525–528; Bazendale et al. in (1990) *Advances in Prostaglandin, Thromboxane and Leukotriene Research*, Vol. 21, Samuelsson et al., eds., Raven Press, NY, pp 302–306].

b. Assays

(1) Receptor Binding Assays

Scintillating matrices with memories beads can be used, for example, in assays screening test compounds as agonists or antagonists of receptors or ion channels or other such cell surface protein. Test compounds of interest are synthesized on the beads or linked thereto, the identity of the linked compounds is encoded in the memory either during or following synthesis, linkage or coating. The scintillating matrices with memories are then incubated with radiolabeled [^{125}I, ^{3}H, or other suitable radiolabel] receptor of interest and counted in a liquid scintillation counter. When radiolabeled receptor binds to any of the structure(s) synthesized or linked to the bead, the radioisotope is in sufficient proximity to the bead to stimulate the scintillant to emit light. In contrast, if a receptor does not bind, less or no radioactivity is associated with the bead, and consequently less light is emitted. Thus, at equilibrium, the presence of

molecules that are able to bind the receptor may be detected. When the reading is completed, the memory in each bead that emits light [or more light than a control] queried and the host computer, decoder/encoder, or scanner can interpret the memory in the bead and identify the active ligand.

(a) Multi-ligand Assay

Mixtures of scintillating matrices with memories with a variety of linked ligands, which were synthesized on the matrices or linked thereto and their identities encoded in each memory, are incubated with a single receptor. The memory in each light-emitting scintillating matrix with memory is queried and the identity of the binding ligand is determined.

(b) Multi-receptor Assays

Similar to conventional indirect or competitive receptor binding assays that are based on the competition between unlabelled ligand and a fixed quantity of radiolabeled ligand for a limited number of binding sites, the scintillating matrices with memories permit the simultaneous screening of a number of ligands for a number of receptor subtypes.

Mixtures of receptor coated beads [one receptor type/per bead; each memory encoded with the identity of the linked receptor] are reacted with labeled ligands specific for each receptor. After the reaction has reached equilibrium, all beads that emit light are reacted with a test compound. Beads that no longer emit light are read.

For example receptor isoforms, such as retinoic acid receptor isoforms, are each linked to a different batch of scintillating matrix with memory beads, and the identity of each isoform is encoded in the memories of linked matrices. After addition of the radiolabeled ligand(s), such as ^{3}H-retinoic acid, a sample of test compounds [natural, synthetic, combinatorial, etc.] is added to the reaction mixture, mixed and incubated for sufficient time to allow the reaction to reach equilibrium. The radiolabeled ligand binds to its receptor, which has been covalently linked to the bead and which the emitted short range electrons will excite the fluophor or scintillant in the beads, producing light. When unlabelled ligand from test mixture is added, if it displaces the labeled ligand it will diminish or stop the fluorescent light signal. At the end of incubation period, the tube can be measured in a liquid scintillation counter to demonstrate if any of the test material reacted with receptor family. Positive samples [reduced or no fluorescence] will be further analyzed for receptor subtyping by querying their memories with the RF detector. In preferred embodiments, each bead will be read and with a fluorescence detector and RF scanner. Those that have a reduced fluorescent signal will be identified and the linked receptor determined by the results from querying the memory.

The same concept can be used to screen for ligands for a number of receptors. In one example, FGF receptor, EGF receptor, and PDGF receptor are each covalently linked to a different batch of scintillating matrix with memory beads. The identity of each receptor is encoded in each memory. After addition of the ^{125}I-ligands [^{125}I-FGF, ^{125}I-EGF, and ^{125}I-PDGF] a sample of test compounds [natural, synthetic, combinatorial, etc.) is added to the tube containing ^{125}I-ligand-receptor-beads, m mixed and incubated for sufficient time to allow the reaction to reach equilibrium. The radiolabeled ligands bind to their respective receptors receptor that been covalently linked to the bead. By virtue of proximity of the label to the bead, the emitted short range electrons will excite the fluophor in the beads. When unlabelled ligand from test mixture is added, if it displaces the any of the labeled ligand it will diminish or stop the fluorescent signal. At the end of incubation period, the tube can be measured in a liquid scintillation counter to demonstrate if any of the test material reacted with the selected receptor family. Positive samples will be further analyzed for receptor type by passing the resulting complexes measuring the fluorescence of each bead and querying the memories by exposing them to RF or the selected EM radiation. The specificity of test ligand is determined by identifying beads with reduced fluorescence that and determining the identity of the linked receptor by querying the memory.

(c) Other Formats

Microspheres, generally polystyrene typically about 0.3 μm–3.9 μm, are synthesized with scintillant inside can either be purchased or prepared by covalently linking scintillant to the monomer prior to polymerization of the polystyrene or other material. They can then be derivatized [or purchased with chemical functional groups], such as —COOH, and —CH_2OH. Selected compounds or libraries are synthesized on the resulting microspheres linked via the functional groups, as described herein, or receptor, such as radiolabeled receptor, can be coated on the microsphere. The resulting "bead" with linked compounds, can used in a variety of SPA and related assays, including immunoassays, receptor binding assays, protein:protein interaction assays, and other such assays in which the ligands linked to the scintillant-containing microspheres are reacted with memories with matrices that are coated with a selected receptor.

For example, ^{125}I-labeled receptor is passively coated on the memory with matrix and then mixed with ligand that is linked to a the scintillant-containing microspheres. Upon binding the radioisotope into is brought into close proximity to the scintillant in which effective energy transfer from the β particle will occur, resulting in emission of light.

Alternatively, the memory with matrix [containing scintillant] can also be coated with ^{3}H-containing polymer on which the biological target [i.e., receptor, protein, antibody, antigen] can be linked [via adsorption or via a functional group]. Binding of the ligand brings the scintillant into close proximity to the label, resulting in light emission.

(2) Cell-based Assays

Cell-based assays, which are fundamental for understanding of the biochemical events in cells, have been used with increasing frequency in biology, pharmacology, toxicology, genetics, and oncology [see, e.g., Benjamin et al. (1992) *Mol. Cell. Biol.* 12:2730–2738] Such cell lines may be constructed or purchased [see, e.g., the Pro-Tox Kit available from Xenometrix, Boulder Colo.; see, also International PCT Application No. WO 94/7208 cell lines]. Established cell lines, primary cell culture, reporter gene systems in recombinant cells, cells transfected with gene of interest, and recombinant mammalian cell lines have been used to set up cell-based assays. For example Xenometrix, Inc. [Boulder, Colo.] provides kits for screening compounds for toxicological endpoints and metabolic profiles using bacteria and human cell lines. Screening is effected by assessing activation of regulatory elements of stress genes fused to reporter genes in bacteria, human liver or colon cell lines and provide information on the cytotoxicity and permeability of test compounds.

In any drug discovery program, cell-based assays offer a broad range of potential targets as well as information on

cytotoxicity and permeability. The ability to test large numbers of compounds quickly and efficiently provides a competitive advantage in pharmaceutical lead identification.

High throughput screening with cell-based assays is often limited by the need to use separation, wash, and disruptive processes that compromise the functional integrity of the cells and performance of the assay. Homogeneous or mix-and-measure type assays simplify investigation of various biochemical events in whole cells and have been developed using scintillation microplates [see, e.g., International PCT Application No. WO 94/26413, which describes scintillant plates that are adapted for attachment and/or growth of cells and proximity assays using such cells]. In certain embodiment herein, cell lines such as those described in International PCT Application No. WO 94/17208 are be plated on scintillant plates, and screened against compounds synthesized on matrices with memories. Matrices with memories encoded with the identity of the linked molecule will be introduced into the plates, the linkages cleaved and the effects of the compounds assessed. Positive compounds will be identified by querying the associated memory.

The scintillant base plate is preferably optically transparent to selected wavelengths that allow cells in culture to be viewed using an inverted phase contrast microscope, and permit the material to transmit light at a given wavelength with maximum efficiency. In addition the base retains its optical properties even after exposure to incident beta radiation from radioisotopes as well as under stringent radiation conditions required for sterilization of the plates. The base plate can be composed of any such optically transparent material containing scintillant, e.g., a scintillant glass based on lanthanide metal compounds. Typically, the base plate is composed of any plastic material, generally formed from monomer units that include phenyl or naphthyl moieties in order to absorb incident radiation energy from radionuclides which are in close proximity with the surface. Preferably the plastic base plate is composed of polystyrene or polyvinyltoluene, into which the scintillant is incorporated. The scintillant includes, but is not limited to: aromatic hydrocarbons such as p-terphenyl, p-quaterphenyl and their derivatives, as well as derivatives of the oxazoles and 1,3,4-oxadiazoles, such as 2-(4-t-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole and 2,5-diphenyloxazole. Also included in the polymeric composition may be a wavelength shifter such as 1,4-bis(5-phenyl-2-oxazolyl)benzene, 9,10-diphenylanthracene, 1,4-bis(2-methylstyryl)-benzene, and other such compounds. The function of the wavelength shifter is to absorb the light emitted by the scintillant substance and re-emit longer wavelength light which is a better match to the photo-sensitive detectors used in scintillation counters. Other scintillant substances and polymer bodies containing them are known to those of skill in this art [see, e.g., European Patent Application No. 0 556 005 A1].

The scintillant substances can be incorporated into the plastic material of the base by a variety of methods. For example, the scintillators may be dissolved into the monomer mix prior to polymerization, so that they are distributed evenly throughout the resultant polymer. Alternatively the scintillant substances may be dissolved in a solution of the polymer and the solvent removed to leave a homogeneous mixture. The base plate of disc may be bonded to the main body of the well or array of wells, which itself may be composed of a plastic material including polystyrene, polyvinyltoluene, or other such polymers. In the case of the multi-well array, the body of the plate may be made opaque, i.e., non-transparent and internally reflective, in order to completely exclude transmission of light and hence minimize "cross-talk." This is accomplished by incorporating into the plastic at the polymerization stage a white dye or pigment, for example, titanium dioxide. Bonding of the base plate to the main body of the device can be accomplished by any suitable bonding technique, for example, heat welding, injection molding or ultrasonic welding.

For example, a 96-well plate is constructed to the standard dimensions of 96-well microtiter plates 12.8 cm×8.6 cm×1.45 cm with wells in an array of 8 rows of 12 wells each. The main body of the plate is constructed by injection molding of polystyrene containing a loading of white titanium oxide pigment at 12%. At this stage, the wells of the microtiter plate are cylindrical tubes with no closed end. A base plate is formed by injection molding of polystyrene containing 2-(4-t-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole (2%) and 9,10-diphenylanthracene (0.5%). The base plate has been silk screen printed with a grid array to further reduce crosstalk. The base plate is then fused in a separate operation to the body by ultrasonic welding, such that the grid array overlies the portions of the microtiter plate between the wells.

A 24-well device is constructed to the dimensions 12.8×8.6×1.4 cm with 24 wells in an array of 4 rows of 6 wells. The main body of the plate [not including the base of each well] is constructed by injection molding of polystyrene containing 12% white titanium oxide pigment. The base 24 of each well is injection molded with polystyrene containing 2-(4-t-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadizaole [2%] and 9,10-diphenylanthracene [0.5%]. The heat from the injected base plastic results in fusion to the main body giving an optically transparent base to the well.

The plates may contain multiple wells that are continuous or that are each discontinuous from the other wells in the array, or they may be single vessels that have, for example, an open top, side walls and an optically transparent scintillant plastic base sealed around the lower edge of the side walls.

In another format the plate, is a single well or tube. The tube may be constructed from a hollow cylinder made from optically transparent plastic material and a circular, scintillant containing, plastic disc. The two components are welded together so as to form a single well or tube suitable for growing cells in culture. As in the plate format, bonding of the circular base plate to the cylindrical portion is achieved by any conventional bonding technique, such as ultrasonic welding. The single well or tube may be any convenient size, suitable for scintillation counting. In use, the single well may either be counted as an insert in a scintillation vial, or alternatively as an insert in a scintillation vial, or alternatively as an insert in a multi-well plate of a flat bed scintillation counter. In this latter case, the main body of the multi-well plate would need to be opaque for reasons given earlier.

The various formats are selected according to use. They may be used for growing cells and studying cellular biochemical processes in living cells or cell fragments. The 96-well plate is a standard format used in experimental cell biology and one that is suitable for use in a flat bed scintillation counter [e.g., Wallac Microbeta or Packard Top Count]. In the multi-well format, it is an advantage to be able to prevent "cross talk" between different wells of the plate that may be used for monitoring different biological processes using different amounts or types of radioisotope. Therefore the main body of the plate can be made from opaque plastic material. The 24-well plate format is commonly used for cell culture. This type of plate is also suitable

for counting in a flat bed scintillation counter. The dimensions of the wells will be larger.

As an alternative format, the transparent, scintillant containing plastic disc is made to be of suitable dimensions so as to fit into the bottom of a counting vessel. The counting vessel is made from non-scintillant containing material such as glass or plastic and should be sterile in order to allow cells to grow and the corresponding cellular metabolic processes to continue. Cells are first cultured on the disc, which is then transferred to the counting vessel for the purposes of monitoring cellular biochemical processes.

The culture of cells on the scintillation plastic base plate of the wells (or the disc) involves the use of standard cell culture procedures, e.g., cells are cultured in a sterile environment at 37° C. in an incubator containing a humidified 95% air/5% CO_2 atmosphere. Various cell culture media may be used including media containing undefined biological fluids such as fetal calf serum, or media which is fully defined and serum-free. For example, MCDB 153 is a selective medium for the culture of human keratinocytes [Tsao et al. (1982) *J. Cell. Physiol.* 110:219–229].

These plates are suitable for use with any adherent cell type that can be cultured on standard tissue culture plasticware, including culture of primary cells, normal and transformed cells derived from recognized sources species and tissue sources. In addition, cells that have been transfected with the recombinant genes may also be cultured using the invention. There are established protocols available for the culture of many of these diverse cell types [see, e.g., Freshney et al. (1987) *Culture of Animal Cells: A Manual of Basic Technique*, 2nd Edition, Alan R. Liss Inc.]. These protocols may require the use of specialized coatings and selective media to enable cell growth and the expression of specialized cellular functions.

The scintillating base plate or disc, like all plastic tissue culture ware, requires surface modification in order to be adapted for the attachment and/or growth of cells. Treatment can involves the use of high voltage plasma discharge, a well established method for creating a negatively charged plastic surface [see, e.g., Amstein et al. (1975) *J. Clinical Microbiol.* 2:46–54]. Cell attachment, growth and the expression of specialized functions can be further improved by applying a range of additional coatings to the culture surface of the device. These can include: (i) positively or negatively charged chemical coatings such as poly-lysine or other biopolymers [McKeehan et al. (1976) *J. Cell Biol.* 71:727–734 (1976)]; (ii) components of the extracellular matrix including collagen, laminin, fibronectin [see, e.g., Kleinman et al. (1987) *Anal. Biochem.* 166:1–13]; and (iii) naturally secreted extracellular matrix laid down by cells cultured on the plastic surface [Freshney et al. et al. (1987) *Culture of Animal Cells: A Manual of Basic Technique*, 2nd Edition, Alan R. Liss Inc.]. Furthermore, the scintillating base plate may be coated with agents, such as lectins, or adhesion molecules for attachment of cell membranes or cell types that normally grow in suspension. Methods for the coating of plasticware with such agents are known [see, e.g., Boldt et al. (1979) *J. Immunol.* 123:808].

In addition, the surface of the scintillating layer may be coated with living or dead cells, cellular material, or other coatings of biological relevance. The interaction of radiolabeled living cells, or other structures with this layer can be monitored with time allowing processes such as binding, movement to or from or through the layer to be measured.

Virtually all types of biological molecules can be studied. A any molecule or complex of molecules that interact with the cell surface or that can be taken up, transported and metabolized by the cells, can be examined using real time analysis. Examples of biomolecules will include receptor ligands, protein and lipid metabolite precursors (e.g., amino acids, fatty acids), nucleosides and any molecule that can be radiolabeled. This would also include ions such as calcium, potassium, sodium and chloride, that are functionally important in cellular homeostasis, and which exist as radioactive isotopes. Furthermore, viruses and bacteria and other cell types, which can be radiolabeled as intact moieties, can be examined for their interaction with monolayer adherent cells grown in the scintillant well format.

The type of radioactive isotope that can be used with this system will typically include any of the group of isotopes that emit electrons having a mean range up to 2000 μm in aqueous medium. These will include isotopes commonly used in biochemistry such as [^{3}H], [^{125}I], [^{14}C], [^{35}S], [^{45}Ca], [^{33}p, and [^{32}p], but does not preclude the use of other isotopes, such as [^{55}Fe], [^{109}Cd] and [^{51}Cr] that also emit electrons within this range. The wide utility of the invention for isotopes of different emission energy is due to the fact that the current formats envisaged would allow changes to the thickness of the layer containing a scintillant substance, thereby ensuring that all the electron energy is absorbed by the scintillant substance. Furthermore, cross-talk correction software is available which can be utilized with all high energy emitters. Applications using these plates include protein synthesis, Ca^{2+} transport, receptor-ligand binding, cell adhesion, sugar transport and metabolism, hormonal stimulation, growth factor regulation and stimulation of motility, thymidine transport, and protein synthesis.

For use in accord with the methods herein, the scintillant plates can include a memory in each well, or alternatively, memory with matrix-linked compounds will be added to each well. The recording device with memory may be impregnated or encased or placed in wells of the plate, typically during manufacture. In preferred embodiments, however, the memories are added to the wells with adsorbed or linked molecules.

In one embodiment, matrices with memories with linked molecules are introduced into scintillant plates in which cells have been cultured [see, e.g., International PCT Application No. WO 94/26413]. For example, cells will be plated on the transparent scintillant base 96-well microplate that permits examination of cells in culture by inverted phase contrast microscope and permits the material to transmit light at a given wavelength with maximum efficiency. Matrices with memories to which test compounds linked by preferably a photocleaveable linker are added to the wells. The identity of each test compound is encoded in the memory of the matrix during synthesis if the compound is synthesized on the matrix with memory or when the compound is linked to the matrix.

Following addition of matrix with memory to the well and release of chemical entities synthesized on the beads by exposure to light or other procedures, the effects of the chemical released from the beads on the selected biochemical events, such as signal transduction, cell proliferation, protein or DNA synthesis, in the cells can be assessed. In this format receptor binding Such events include, but are not limited to:

whole cell receptor-ligand binding tagonist or antagonist], thymidine or uridine transport, protein synthesis (using, for example, labeled cysteine, methionine, leucine or proline], hormone and growth factor induced stimulation and motility, and calcium uptake.

In another embodiment, the memories are included in the plates either placed in the plates or manufactured in the wells

of the plates. In these formats, the identities of the contents of the well is encoded into the memory. Of course it is understood, that the information encoded and selection of encased or added memories depends upon the selected protocol.

In another format, cells will be plated on the tissue culture plate, after transferring the matrices with memories and release of compounds synthesized on the beads in the well. Cytostatic, cytotoxic and proliferative effects of the compounds will be measured using colorimetric [MTT, XTT, MTS, Alamar blue, and Sulforhodamine B], fluorimetric [carboxyfluorescein diacetate], or chemiluminescent reagents [i.e., CytoLite™, Packard Instruments, which is used in a homogeneous luminescent assay for cell proliferation, cell toxicity and multi-drug resistance].

For example, cells that have been stably or transiently transfected with a specific gene reporter construct containing an inducible promoter operatively linked to a reporter gene that encodes an indicator protein can be calorimetrically monitored for promoter induction. Cells will be plated on the tissue culture 96-well microtiter plate and after addition of memories with matrices in the wells and release of chemical entities synthesized on the matrices, the effect of the compound released from the beads on the gene expression will be assessed. The Cytosensor Microphysiometer [Molecular Devices] evaluates cellular responses that are mediated by G protein-linked receptors, tyrosine kinase-linked receptors, and ligand-gated ion channels. It measures extracellular pH to assess profiles of compounds assessed for the ability to modulate activities of any of the these cell surface proteins by detecting secretion of acid metabolites as a result of altered metabolic states, particularly changes in metabolic rate. Receptor activation requires use of ATP and other energy resources of the cell thereby leading to increased in cellular metabolic rate. For embodiments herein, the memories with matrices, particularly those modified for measuring pH, and including linked test compounds, can be used to track and identify the added test compound added and also to detect changes in pH, thereby identifying linked molecules that modulate receptor activities.

3. Memories With Matrices For Non-radioactive Energy Transfer Proximity Assays

Non-radioactive energy transfer reactions, such as FET or FRET, FP and HTRF assays, are homogeneous luminescence assays based on energy transfer are carried out between a donor luminescent label and an acceptor label [see, e.g., Cardullo et al. (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85:8790–8794; Peerce et al. (1986) *Proc. Natl. Acad. Sci. U.S.A.* 83:8092–8096; U.S. Pat. No. 4,777,128; U.S. Pat. No. 5,162,508; U.S. Pat. No. 4,927,923; U.S. Pat. No. 5,279,943; and International PCT Application No. WO 92/01225]. The donor label is usually a rare earth metal cryptate, particularly europium trisbipyridine diamine [EuTBP] or terbium trisbipyridine diamine [TbTBP] and an acceptor luminescent, presently fluorescent, label. When the donor is EuTBP, the acceptor is preferably allopycocyanin [APC], allophycocyanin B, phycocyanin C or phycocyanin R, and when the donor is TbTBP, the acceptor is a rhodamine, thiomine, phycocyanin R, phycoerythrocyanin, phycoerythrin C, phycoerythrin B or phycoerythrin R.

Energy transfer between such donors and acceptors is highly efficient, giving an amplified signal and thereby improving the precision and sensitivity of the assay. Within distances characteristic of interactions between biological molecules, the excitation of a fluorescent label (donor) is transferred non radiatively to a second fluorescent label (acceptor). When using europium cryptate as the donor, APC, a phycobiliprotein of 5 kDa, is presently the preferred acceptor because it has high molar absorptivity at the cryptate emission wavelength providing a high transfer efficiency, emission in a spectral range in which the cryptate signal is insignificant, emission that is not quenched by presence of sera, and a high quantum yield. When using Eu^{3+} cryptate as donor, an amplification of emitted fluorescence is obtained by measuring APC emission.

The rare earth cryptates are formed by the inclusion of a luminescence lanthanide ion in the cavity of a macropolycyclic ligand containing 2,2'-biphyridine groups as light absorbers [see, e.g., U.S. Pat. No. 5,162,508; U.S. Pat. No. 4,927,923; U.S. Pat. No. 5,279,943; and International PCT Application No. WO 92/01225]. Preferably the $Eu3^{+}$ trisbypryidine diamine derivative, although the acceptor may be used as the label, is cross-linked to antigens, antibodies, proteins, peptides, and oligonucleotides and other molecules of interest.

For use herein, matrices with memories are prepared that incorporate either the donor or, preferably the acceptor, into or on the matrix. In practice, as with the scintillating matrices with memories, the matrices may be of any format, i.e. particulate, or continuous, and used in any assay described above for the scintillating matrices. For example, the recording device is coated with a protective coating, such as glass or polystyrene. If glass it can be etched. As with preparation of the scintillating matrices with memories, compositions containing the donor or preferably acceptor, such as APC, and typically a polymer or gel, are coated on the recording device or the device is mixed with the composition to produce a fluorescing matrix with memory. To make these matrices resistant to chemical reaction, if needed, they may be coated with polymers such as polyvinylbenzene or polystyrene. Molecules, such as the constituents of combinatorial libraries, are synthesized on the fluorescing matrices with memories, or molecules or biological particles are linked thereto, the identity of the synthesized molecules or linked molecules or biological particles is encoded in memory, and the resulting matrices with memories employed in any suitable assay, including any of those described for the scintillating memories with matrices. In particular, these homogeneous assays using long-lived fluorescence rare earth cryptates and amplification by non radiative energy transfer have been adapted to use in numerous assays including assays employing ligand receptor interaction, signal transduction, transcription factors (protein-protein interaction), enzyme substrate assays and DNA hybridization and analysis [see, Nowak (1993) *Science* 270:368; see, also, Velculescu et al. (1995) *Science* 270:484–487, and Schena et al. (1995) *Science* 270:467–470, which describe methods quantitative and simultaneous analysis of a large number of transcripts that are particularly suited for modification using matrices with memories]. Each of these assays may be modified using the fluorescing matrices with memories provided herein.

For example, a receptor will be labeled with a europium cryptate [where the matrices with memories incorporate, for example allophycocyanin (APC)] or will be labeled with APC, where the matrices incorporate a europium cryptate. After mixing receptor and mixtures of matrices with different ligands, the mixture is exposed to laser excitation at 337 nm, and, if reaction has occurred, typical signals of europium cryptate and APC over background are emitted. Measurement with an interference filter centered at 665 nm selects the signal of the APC labeled receptor from that of europium cryptate labeled ligand on the beads. If particulate,

the memories of matrices that emit at 665, can be queried to identify linked ligands.

4. Other Applications Using Memories With Matrices and Luminescing Memories With Matrices

a. Natural Product Screening

In the past, the vast majority of mainline pharmaceuticals have been isolated form natural products such as plants, bacteria, fungus, and marine microorganisms. Natural products include microbials, botanicals, animal and marine products. Extracts of such sources are screened for desired activities and products. Selected products include enzymes [e.g., hyaluronidase], industrial chemicals [e.g., petroleum emulsifying agents], and antibiotics [e.g., penicillin]. It is generally considered that a wealth of new agents still exist within the natural products pool. Large mixtures of natural products, even within a fermentation broth, can be screened using the matrices with memory combinations linked, for example, to peptides, such as antigens or antibody fragments or receptors, of selected and known sequences or specificities, or to other biologically active compounds, such as neurotransmitters, cell surface receptors, enzymes, or any other identified biological target of interest. Mixtures of these peptides linked to memory matrices can be introduced into the natural product mixture. Individual binding matrices, detected by an indicator, such as a fluorometric dye, can be isolated and the memory queried to determine which linked molecule or biological particle is bound to a natural product.

b. Immunoassays and Immunodiagnostics

The combinations and methods provided herein represent major advances in immunodiagnostics. Immunoassays [such as ELISAs, RIAs and EIAs (enzyme immunoassays)] are used to detect and quantify antigens or antibodies.

(1) Immunoassays

Immunoassays detect or quantify very small concentrations of analytes in biological samples. Many immunoassays use solid supports in which antigen or antibody is covalently, non-covalently, or otherwise, such as via a linker, attached to a solid support matrix. The support-bound antigen or antibody is then used as an analyte in the assay. As with nucleic acid analysis, the resulting antibody-antigen complexes or other complexes, depending upon the format used, rely on radiolabels or enzyme labels to detect such complexes.

The use of antibodies to detect and/or quantitate reagents ["antigens"] in blood or other body fluids has been widely practiced for many years. Two methods have been most broadly adopted. The first such procedure is the competitive binding assay, in which conditions of limiting antibody are established such that only a fraction [usually 30–50%] of a labeled [e.g., radioisotope, fluophore or enzyme] antigen can bind to the amount of antibody in the assay medium. Under those conditions, the addition of unlabeled antigen [e.g., in a serum sample to be tesied] then competes with the labeled antigen for the limiting antibody binding sites and reduces the amount of labeled antigen that can bind. The degree to which the labeled antigen is able to bind is inversely proportional to the amount of unlabeled antigen present. By separating the antibody-bound from the unbound labeled antigen and then determining the amount of labeled reagent present, the amount of unlabeled antigen in the sample [e.g., serum] can be determined.

As an alternative to the competitive binding assay, in the labeled antibody, or "immunometric" assay [also known as "sandwich" assay], an antigen present in the assay fluid is specifically bound to a solid substrate and the amount of antigen bound is then detected by a labeled antibody [see, e.g., Miles et al. (1968) *Nature* 29:186–189; U.S. Pat. No. 3,867,517; U.S. Pat. No. 4,376,110]. Using monoclonal antibodies two-site immunometric assays are available [see, e.g., U.S. Pat. No. 4,376,110]. The "sandwich" assay has been broadly adopted in clinical medicine. With increasing interest in "panels" of diagnostic tests, in which a number of different antigens in a fluid are measured, the need to carry out each immunoassay separately becomes a serious limitation of current quantitative assay technology.

Some semi-quantitative detection systems have been developed [see, e.g., Buechler et al. (1992) *Clin. Chem.* 38:1678–1684; and U.S. Pat. No. 5,089,391] for use with immunoassays, but no good technologies yet exist to carefully quantitate a large number of analytes simultaneously [see, e.g., Ekins et al. (1990) *J. Clin. Immunoassay* 13:169–181] or to rapidly and conveniently track, identify and quantitate detected analytes.

The methods and memories with matrices provided herein provide a means to quantitate a large number of analytes simultaneously and to rapidly and conveniently track, identify and quantitate detected analytes.

(2) Multianalyte Immunoassays

The combinations of matrix with memories provided herein permits the simultaneous assay of large numbers of analytes in any format. In general, the sample that contains an analyte, such as a ligand or any substance of interest, to be detected or quantitated, is incubated with and bound to a protein, such as receptor or antibody, or nucleic acid or other molecule to which the analyte of interest binds. In one embodiment, the protein or nucleic acid or other molecule to which the analyte of interest binds has been linked to a matrix with memory prior to incubation; in another embodiment, complex of analyte or ligand and protein, nucleic acid or other molecule to which the analyte of interest binds is linked to the matrix with memory after the incubation; and in a third embodiment, incubation to form complexes and attachment of the complexes to the matrix with memory are simultaneous. In any embodiment, attachment is effected, for example, by direct covalent attachment, by kinetically inert attachment, by noncovalent linkage, or by indirect linkage, such as through a second binding reaction [i.e., biotin-avidin, Protein A-antibody, antibody-hapten, hybridization to form nucleic acid duplexes of oligonucleotides, and other such reactions and interactions]. The complexes are detected and quantitated on the solid phase by virtue of a label, such as radiolabel, fluorescent label, luminophore label, enzyme label or any other such label. The information that is encoded in the matrix with memory depends upon the selected embodiment. If, for example, the target molecule, such as the protein or receptor is bound to the solid phase, prior to complexation, the identity of the receptor and/or source of the receptor may be encoded in the memory in the matrix.

For example, the combinations provided herein are particularly suitable for analyses of multianalytes in a fluid, and particularly for multianalyte immunoassays. In one example, monoclonal antibodies very specific for carcinoembryonic antigen [CEA], prostate specific antigen [PSA], CA-125, alphafetoprotein [AFP], TGF-β, IL-2, IL-8 and IL-10 are each covalently attached to a different batch of

matrices with memories using well-established procedures and matrices for solid phase antibody assays. Each antibody-matrix with memory complex is given a specific identification tag, as described herein.

A sample of serum from a patient to be screened for the presence or concentration of these antigens is added to a tube containing two of each antibody-matrix with memory complex [a total of 16 beads, or duplicates of each kind of bead]. A mixture of monoclonal antibodies, previously conjugated to fluorescent dyes, such as fluorescein or phenyl-EDTA-Eu chelate, reactive with different epitopes on each of the antigens is then added. The tubes are then sealed and the contents are mixed for sufficient time [typically one hour] to allow any antigens present to bind to their specific antibody-matrix with memory-antigen complex to produce antibody-matrix with memory-antigen-labeled antibody complexes. At the end of the time period, these resulting complexes are briefly rinsed and passed through an apparatus, such as that set forth in FIG. 7, but with an additional light source. As each complex passes through a light source, such as a laser emitting at the excitation wavelength of fluorescein, about 494 nm, or 340 nm for the Eu chelate complex, its fluorescence is measured and quantitated by reading the emitted photons at about 518 nm for fluorescein or 613 nm for phenyl-EDTA-Eu, and as its identity is determined by the specific signal received by the RF detector. In this manner, eight different antigens are simultaneously detected and quantitated in duplicate.

In another embodiment, the electromagnetically tagged matrices with recorded information regarding linked antibodies can be used with other multianalyte assays, such as those described by Ekins et al. [(1990) *J. Clin. Immunoassay* 13:169–181; see, also International PCT Applications Nos. 89/01157 and 93/08472, and U.S. Pat. Nos. 4,745,072, 5,171,695 and 5,304,498]. These methods rely on the use of small concentrations of sensor-antibodies within a few μm^2 area. Individual memories with matrices, or an array of memories embedded in a matrix are used. Different antibodies are linked to each memory, which is programmed to record the identity of the linked antibody. Alternatively, the antibody can be linked, and its identity or binding sites identified, and the information recorded in the memory. Linkage of the antibodies can be effected by any method known to those of skill in this art, but is preferably effected using cobalt-iminodiacetate coated memories [see, Hale (1995) *Analytical Biochem.* 231:46–49, which describes means for immobilization of antibodies to cobalt-iminodiacetate resin] mediated linkage particularly advantageous. Antibodies that are reversibly bound to a cobalt-iminodiacetate resin are attached in exchange insert manner when the cobalt is oxidized from the +2 to +3 state. In this state the antibodies are not removed by metal chelating regents, high salt, detergents or chaotropic agents. They are only removed by reducing agents. In addition, since the metal binding site in antibodies is in the C-terminus heavy chain, antibodies so-bound are oriented with the combining site directed away from the resin.

In particular antibodies are linked to the matrices with memories. The matrices are either in particular form or in the form of a slab with an array of recording devices linked to the matrices or microtiter dish or the like with a recording device in each well. Antibodies are then linked either to each matrix particle or to discrete "microspots" on the slab or in the microtiter wells. In one application, prior to use of these matrices with memories, they are bound to a relatively low affinity anti-idiotype antibody [or other species that specifically recognizes the antibody binding site, such as a single chain antibody or peptidomimetic] labeled with a fluophore [e.g., Texas Red, acridine, fluorescein, ellipticine, rhodamine, Lissamine rhodamine B, Malachite Green, erythrosin, tetramethylrhodamine, eosin, pyrene, anthracene, methidium, ethydium, phenanthroline, 4-dimethylaminonaphthalene, quinoxaline, 2-dimethylaminonaphthalene, 7-dimethylamino-4-methylcoumarin, 7-dimethylaminocoumarin, 7-hydroxy-4-methylcoumarin, 7-hydroxycoumarin, 7-methoxycoumarin, 7-acetoxycoumarin, 7-diethylamino-3-phenyl-4-methyl-coumarin, isoluminol, benzophenone, dansyl, dabsyl, mansyl, sulfo rhodamine, 4-acetamido-4'-stilbene-2,2'-disulfonic acid disodium salt, 4-benzamido-4'-stilbene-2,2'-disulfonic acid disodium salt] to measure the concentration of and number of available binding sites present on each matrix with memory particle or each microspot, which information is then encoded into each memory for each microspot or each particle [see, Ekins et al. (1990) *J. Clin. Immunoassay* 13:169–181]. These low affinity antibodies are then eluted, and the matrices can be dried and stored until used.

Alternatively or additionally, the memories in the particles or at each microspot could be programmed with the identity or specificity of the linked antibody, so that after reaction with the test sample and identification of complexed antibodies, the presence and concentration of particular analytes in the sample can be determined. They can be used for multianalyte analyses as described above.

After reaction with the test sample, the matrices with memories are reacted with a second antibody, preferably, although not necessarily, labeled with a different label, such as a different fluophore, such as fluorescein. After this incubation, the microspots or each matrix particle is read by passing the particle through a laser scanner [such as a confocal microscope, see, e.g., Ekins et al. (1990) *J. Clin. Immunoassay* 13:169–181; see also U.S. Pat. No. 5,342,633] to determine the fluorescence intensity. The memories at each spot or linked to each particle are queried to determine the total number of available binding sites, thereby permitting calculation of the ratio of occupied to unoccupied binding sites.

Equilibrium dialysis and modifications thereof has been used to study the interaction of antibody or receptor or other protein or nucleic acid with low molecular weight dialyzable molecules that bind to the antibody or receptor or other protein or nucleic acid. For applications herein, the antibody, receptor, protein or nucleic acid is linked to solid support (matrix with memory) and is incubated with the ligand.

In particular, this method may be used for analysis of multiple binding agents [receptors], linked to matrices with memories, that compete for available ligand, which is present in limiting concentration. After reaction, the matrices with memories linked to the binding agents [receptors] with the greatest amount of bound ligand, are the binding agents [receptors] that have the greatest affinity for the ligand.

The use of matrices with memories also permits simultaneous determination of K_a values of multiple binding agents [receptors] or have multiple ligands. For example, a low concentration of labeled ligand is mixed with a batch of different antibodies bound to matrices with memories. The mixture is flowed through a reader [i.e., a Coulter counter or other such instrument that reads RF and the label] could simultaneously measure the ligand [by virtue of the label] and identity of each linked binding agent for linked ligand] as the chip is read. After the reaction equilibrium

[determined by monitoring progress of the reaction] labeled ligand is added and the process of reading label and the chips repeated. This process is repeated until all binding sites on the binding agent [or ligand] approach saturation, thereby permitting calculation of K_a values and binding sites that were available.

C. Selection of Antibodies and Other Screening Methods

(1) Antibody Selection

In hybridoma preparation and selection, fused cells are plated into, for example, microtiter wells with the matrices with memory-tagged antibody binding reagent [such as protein A or Co-chelate [see, e.g., Smith et al. (1992) *Methods: A Companion to Methods in Enzymology* 4, 73 (1992); III et al. (1993) *Biophys J.* 64:919; Loetscher et al. (1992) *J. Chromatography* 595:113–199; U.S. Pat. No. 5,443,816; Hale (1995) *Analytical Biochem.* 231:46–49]. The solid phase is removed, pooled and processed batchwise to identify the cells that produce antibodies that are the greatest binders [see, e.g., U.S. Pat. No. 5,324,633 for methods and device for measuring the binding affinity of a receptor to a ligand; or the above method by which phage libraries are screened for highest K_A phage, i.e., limiting labeled antigen].

(2) Antibody Panning

Memories with matrices with antibody attached thereto [e.g. particularly embodiments in which the matrix is a plate] may be used in antibody panning [see, e.g., Wysocki et al. (1978) *Proc. Natl. Acad. Sci. U.S.A.* 75:2844–48; Basch et al. (1983) *J. Immunol. Methods* 56:269; Thiele et al. (1986) *J. Immunol.* 136:1038–1048; Mage et al. (1981) *Eur. J. Immunol.* 11:226; Mage et al. (1977) *J. Immunol. Methods* 15:47–56; see, also, U.S. Pat. Nos. 5,217,870 and 5,087,570, for descriptions of the panning method]. Antibody panning was developed as a means to fractionate lymphocytes on the basis of surface phenotype based on the ability of antibody molecules to adsorb onto polystyrene surfaces and retain the ability to bind antigen. Originally [Wysocki et al. (1978) *Proc. Natl. Acad. Sci. U.S.A.* 75:2844–2848] polystyrene dishes coated with antibodies specific for cell surface antigens and permit cells to bind to the dishes, thereby fractionating cells. In embodiments herein, polystyrene or other suitable matrix is associated with a memory device and coated with an antibody, whose identity is recorded in the memory. Mixtures of these antibody coated memories with matrices can be mixed with cells, and multiple cell types can be sorted and identified by querying the memories to which cells have bound.

d. Phage Display

Phage, viruses, bacteria and other such manipulable hosts and vectors [referred to as biological particles] can be modified to express selected antigens [peptides or polypeptides] on their surfaces by, for example, inserting DNA encoding the antigen into the host or vector genome, at a site such as in the DNA encoding the coat protein, such that upon expression the antigen [peptide or polypeptide] is presented on the surface of the virus, phage or bacterial host. Libraries of such particles that express diverse or families of proteins on their surfaces have been prepared. The resulting library is then screened with a targeted antigen [receptor or ligand] and those viruses with the highest affinity for the targeted antigen [receptor or ligand] are selected [see, e.g., U.S. Pat. Nos. 5,403,484, 5,395,750, 5,382,513, 5,316,922, 5,288,622, 5,223,409, 5,223,408 and 5,348,867].

Libraries of antibodies expressed on the surfaces of such packages have been prepared from spleens of immunized and unimmunized animals and from humans. In the embodiment in which a library of phage displaying antibodies from unimmunized human spleens is prepared, it is often of interest to screen this library against a large number of different antigens to identify a number of useful human antibodies for medical applications. Phage displaying antibody binding sites derived from single or small numbers of spleen cells can be separately produced, expanded into large batches, and bound to matrices with memories, such as programmable PROM or EEPROM memories, and identified according to phage batch number recorded in the memory. Each antigen can then be exposed to a large number of different phage-containing memory devices, and those that bind the antigen can be identified by one of several means, including radiolabeled, fluorescent labeled, enzyme labeled or alternate (e.g., mouse) tagged antibody labeled antigen. The encoded information in the thus identified phage-containing devices, relates to the batch of phage reactive with the antigen.

Libraries can also be prepared that contain modified binding sites or synthetic antibodies. DNA molecules, each encoding proteins containing a family of similar potential binding domains and a structural signal calling for the display of the protein on the outer surface of a selected viral or bacterial or other package, such as a bacterial cell, bacterial spore, phage, or virus are introduced into the bacterial host, virus or phage. The protein is expressed and the potential binding domain is displayed on the outer surface of the particle. The cells or viruses bearing the binding domains to which target molecules bind are isolated and amplified, and then are characterized. In one embodiment, one or more of these successful binding domains is used as a model for the design of a new family of potential binding domains, and the process is repeated until a novel binding domain having a desired affinity for the target molecule is obtained. For example, libraries of de novo synthesized synthetic antibody library containing antibody fragments expressed on the surface have been prepared. DNA encoding synthetic antibodies, which have the structure of antibodies, specifically Fab or Fv fragments, and contain randomized binding sequences that may correspond in length to hypervariable regions [CDRs] can be inserted into such vectors and screened with an antigen of choice.

Synthetic binding site libraries can be manipulated and modified for use in combinatorial type approaches in which the heavy and light chain variable regions are shuffled and exchanged between synthetic antibodies in order to affect specificities and affinities. This enables the production of antibodies that bind to a selected antigen with a selected affinity. The approach of constructing synthetic single chain antibodies is directly applicable to constructing synthetic Fab fragments which can also be easily displayed and screened. The diversity of the synthetic antibody libraries can be increased by altering the chain lengths of the CDRs and also by incorporating changes in the framework regions that may affect antibody affinity. In addition, alternative libraries can be generated with varying degrees of randomness or diversity by limiting the amount of degeneracy at certain positions within the CDRs. The synthetic binding site can be modified further by varying the chain lengths of the CDRs and adjusting amino acids at defined positions in the CDRs or the framework region which may affect affinities. Antibodies identified from the synthetic antibody library can

easily be manipulated to adjust their affinity and or effector functions. In addition, the synthetic antibody library is amenable to use in other combinatorial type approaches. Also, nucleic acid amplification techniques have made it possible to engineer humanized antibodies and to clone the immunoglobulin [antibody] repertoire of an immunized mouse from spleen cells into phage expression vectors and identify expressed antibody fragments specific to the antigen used for immunization [see, e.g., U.S. Pat. No. 5,395,750].

The phage or other particles, containing libraries of modified binding sites, can be prepared in batches and linked to matrices that identify the DNA that has been inserted into the phage. The matrices are then mixed and screened with labeled antigen [e.g., fluorescent or enzymatic] or hapten, using an assay carried out with limiting quantities of the antigen, thereby selecting for higher affinity phage. Thus, libraries of phage linked to matrix particles with memories can be prepared. The matrices are encoded to identify the batch number of the phage, a sublibrary, or to identify a unique sequence of nucleotides or amino acids in the antibody or antibody fragment expressed on its surface. The library is then screened with labeled antigens. The antigens are labeled with enzyme labels or radiolabels or with the antigen bound with a second binding reagent, such as a second antibody specific for a second epitope to which a fluorescent antigen binds.

Following identification of antigen bound phage, the matrix particle can be queried and the identity of the phage or expressed surface protein or peptide determined. The resulting information represents a profile of the sequence that binds to the antigen. This information can be analyzed using methods known to those of skill in this art.

e. Anti-microbial Assays and Mutagenicity Assays

Compounds are synthesized or linked to matrix with memory. The linkage is preferably a photocleavable linkage or other readily cleavable linkage. The matrices with memories with linked compounds, whose identities are programmed into each memory are the placed on, for example, 10-cm culture plates, containing different bacteria, fungi, or other microorganism. After release of the test compounds the anti-microbial effects of the chemical will be assessed by looking for lysis or other indicia of anti-microbial activity. In preferred embodiments, arrays of memories with matrices can be introduced into plates. The memories are encoded with the identity of -the linked or associated test compound and the position on the array.

The AMES test is the most widely used mutagen/carcinogen screening assay [see, e.g., Ames et al. (1975) *Mutation Res.* 31:347–364; Ames et al. (1973) *Proc. Natl. Acad. Sci. U.S.A.* 70:782–786.; Maron et al., (1983) *Mutation Research* 113:173; Ames (1971) in *Chemical Mutagens, Principles and Methods for their Detection*, Vol. 1, Plenum Press, NY, pp 267–282]. This test uses several unique strains of Salmonella typhimurium that are histidine-dependent for growth and that lack the usual DNA repair enzymes. The frequency of normal mutations that render the bacteria independent of histidine [i.e., the frequency of spontaneous revertants] is low. The test evaluates the impact of a compound on this revertant frequency. Because some substances are converted to a mutagen by metabolic action, the compound to be tested is mixed with the bacteria on agar plates along with the liver extract. The liver extract serves to mimic metabolic action in an animal. Control plates have only the bacteria and the extract. The mixtures are allowed to incubate. Growth of bacteria is checked by counting colonies. A test is positive where the number of colonies on the plates with mixtures containing a test compound significantly exceeds the number on the corresponding control plates.

A second type of Ames test [see, International PCT Application No. WO 95/10629, which is based on U.S. application Ser. No. 08/011,617; and Gee et al. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91:11606–11610; commercially avail from Xenometrix, Boulder Colo.] is of interest herein. This test provides a panel of *Salmonella typhimurium* strains for use as a detection system for mutagens that also identifies mutagenic changes. Although a direct descendant of the traditional Ames Salmonella reverse mutation assay in concept, the Ames II assay provides the means to rapidly screen for base mutations through the use of a mixture of six different Salmonella strains.

These new strains carry his mutations listed in the table below. All are deleted for uvrB and are deficient therefore in excision repair. In addition, all six have lipopolysaccharide [rfa] mutations rendering them more permeable, and all contain the pKM^{101} plasmid conferring enhanced mutability.

STRAIN	BASE CHANGE	MUTATION
TA7001	A:T → G:C	hisG1775
TA7002	T:A → A:T	hisC9138
TA7003	T:A → G:C	hisG9074
TA7004	G:C → A:T	hisG9133
TA7005	G:C → A:T	hisG9130
TA7006	G:C → C:G	hisC9070

These strains, which revert at similar spontaneous frequencies [approximately 1 to 10×10^8] can be exposed and plated separately for determining mutational spectra, or mixed and exposed together to assess broad mutagenic potential. The assay takes 3 days from start to finish and can be performed in 96 well- or 384 well-microtiter plates. Revertant colonies are scored using bromo-creosol purple indicator dye in the growth medium. The mixed strains can be assayed first as part of a rapid screening program. Since this six strain mixture is slightly less sensitive than individual strains tested alone, compounds which are negative for the mix can be retested using all six strains. For all but the weakest mutagens, the Ames II strain mixture appears to be capable of detecting reversion events even if only one strain is induced to revert. The mixed strains provide a means to perform rapid initial screening for genotoxins, while the battery of base-specific tester strains permit mutational spectra analysis.

As modified herein, the test compounds are linked to matrices with memories, that have been encoded with the identity of the test compounds. The assays can be performed on multiple test compounds simultaneously using arrays of matrices with memories or multiple matrices with memories encoded with the identity of the linked test compound and the array position or plate number into which the compound is introduced.

f. Hybridization Assays and Reactions

(1) Hybridization Reactions

It is often desirable to detect or quantify very small concentrations of nucleic acids in biological samples. Typically, to perform such measurements, the nucleic acid in the sample [i.e., the target nucleic acid] is hybridized to a detection oligonucleotide. In order to obtain a detectable

signal proportional to the concentration of the target nucleic acid, either the target nucleic acid in the sample or the detection oligonucleotide is associated with a signal generating reporter element, such as a radioactive atom, a chromogenic or fluorogenic molecule, or an enzyme [such as alkaline phosphatasel that catalyzes a reaction that produces a detectable product. Numerous methods are available for detecting and quantifying the signal.

Following hybridization of a detection oligonucleotide with a target, the resulting signal-generating hybrid molecules must be separated from unreacted target and detection oligonucleotides. In order to do so, many of the commonly used assays immobilize the target nucleic acids or detection oligonucleotides on solid supports. Presently available solid supports to which oligonucleotides are linked include nitrocellulose or nylon membranes, activated agarose supports, diazotized cellulose supports and non-porous polystyrene latex solid microspheres. Linkage to a solid support permits fractionation and subsequent identification of the hybridized nucleic acids, since the target nucleic acid may be directly captured by oligonucleotides immobilized on solid supports. More frequently, so-called "sandwich" hybridization systems are used. These systems employ a capture oligonucleotide covalently or otherwise attached to a solid support for capturing detection oligonucleotide-target nucleic acid adducts formed in solution [see, e.g., , EP 276,302 and Gingeras et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:1173]. Solid supports with linked oligonucleotides are also used in methods of affinity purification. Following hybridization or affinity purification, however, if identification of the linked molecule or biological material is required, the resulting complexes or hybrids or compounds must be subjected to analyses, such as sequencing. The combinations and methods herein eliminate the need for such analyses.

Use of matrices with memories in place of the solid support matrices used in the prior hybridization methods permits rapid identification of hybridizing molecules. The identity of the linked oligonucleotide is written or encoded into the memory. After reaction, hybrids are identified, such as by radioactivity or separation, and the identify of hybridizing molecules are determined by querying the memories.

(2) Hybridization Assays

Mixtures nucleic acid probes linked to the matrices with memories can be used for screening in assays that heretofore had to be done with one probe at a time or with mixtures of probes followed by sequencing the hybridizing probes. There are numerous examples of such assays [see, e.g., U.S. Pat. No. 5,292,874, "Nucleic acid probes to *Staphylococcus aureus*" to Milliman, and U.S. Pat. No. 5,232,831, "Nucleic acid probes to *Streptococcus Dyopenes*" to Milliman, et al.; see, also, U.S. Pat. Nos. 5,216,143, 5,284,747 5,352,579 and 5,374,718]. For example, U.S. Pat. No. 5,232,831 provides probes for the detection of particular Streptococcus species from among related species and methods using the probes. These probes are based on regions of Streptococcus rRNA that are not conserved among related Streptococcus species. Particular species are identified by hybridizing with mixtures of probes and ascertaining which probe(s) hybridize. By virtue of the instant matrices with memories, following hybridization, the identity of the hybridizing probes can be determined by querying the memories, and thereby identifying the hybridizing probe.

9. Combinatorial Libraries and Other Libraries and Screening Methododologies

The combinations of matrices with memories are applicable to virtually any synthetic scheme and library preparation and screening protocol. These include, those discussed herein, and also methodologies and devices, such as the Chiron "pin" technology [see, e.g., International PCT application No. WO 94/11388; Geysen et al. (1985) *Proc. Natl. Acad. Sci. U.S.A.* 82:178; Geysen et al. (1987) *J. Immunol. Meth.* 102:259–274; Maeji et al. (1994) *Reactive Polymers* 22:203–212], which relies on a support composed of annular synthesis components that have an active surface for synthesis of a modular polymer and an inert support rod that is positioned axially to the annular synthesis components. This pin technology was developed for the simultaneous synthesis of multiple peptides. In particular the peptides are synthesized on polyacrylic acid grafted on the tip of polyethylene pins, typically arranged in a microtiter format. Amino acid coupling is effected by immersing the pins in a microtiter plate. The resulting peptides remain bound to the pins and can be reused.

As provided herein, "pins" may be linked to a memory or recording device, preferably encasing the device, or each pin may be coded and the code and the identity of the associated linked molecule(s) stored in a remote memory. As a result it will not be necessary to physically array the pins, rather the pins can be removed and mixed or sorted.

Also of interest herein, are DIVERSOMER™ technology libraries produced by simultaneous parallel synthesis schemes for production of nonoligomeric chemical diversity [see, e.g., U.S. Pat. No. 5,424,483; Hobbs DeWitt et al. (1994) *Drug Devel. Res.* 33:116–124; Czarnik et al. (1994) *Polym. Prepr.* 35:985; Stankovic et al. (1994) in *Innovation Perspect. Solid Phase Synth. Collect. Pap., Int. Symp.*, 3rd Epton, R. (Ed), pp. 391–6; DeWitt et al. (1994) *Drug Dev. Res.* 33:116–124; Hobbs DeWitt et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909–6913]. In this technology a starting material is bonded to a solid phase, such as a matrix material, and is subsequently treated with reagents in a stepwise fashion. Because the products are linked to the solid support, multistep syntheses can be automated and multiple reactions can be performed simultaneously to produce libraries of small molecules. This technology can be readily improved by combining the matrices with memories or encoding the matrix supports in accord with the methods herein.

The matrices with memories, either those with memories in proximity or those in which the matrix includes a code stored in a remote memory, can be used in virtually any combinatorial library protocol. These protocols or methodologies and libraries, include but are not limited to those described in any of following references: Zuckermann et al. (1994) *J. Med. Chem.* 37:2678; Martin et al. (1995) *J. Med. Chem.* 38:1431; Campbell et al. (1995) *J. Am. Chem. Soc.* 117:5381; Salmon et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:11708; Patek et al. (1994) Tetrahedron Lett. 35:9169; Patek et al. (1995) *Tetrahedron Lett.* 36:2227; Hobbs DeWitt et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6906; Baldwin et al. (1995) *J. Am. Chem. Soc.* 117:5588; and others.

h. Nucleic Acid Sequencing

Methods of DNA sequencing based on hybridization of DNA fragments with a complete set of fixed length oligonucleotides [usually 8-mers] that are immobilized individually as dots in a 2-dimensional matrix is sufficient for computer-assisted reconstruction of the sequences of fragments up to 200 bases long [International PCT Application WO 92/10588]. The nucleic acid probes are of a length shorter than a target, which is hybridized to the probes under conditions such that only those probes having an exact

complementary sequence are hybridized maximally, but those with mismatches in specific locations hybridize with a reduced affinity, as can be determined by conditions necessary to dissociate the pairs of hybrids. Alignment of overlapping sequences from the hybridizing probes reconstructs the complement of the target [see, EP 0 535 242 Al, International PCT Application WO 95/00530, and Khrapko et al. (1989) *FEBS Ltrs.* 256:118–122]. The target fragment with the sequence of interest is hybridized, generally under highly stringent conditions that tolerate no mismatches or as described below a selected number of mismatches, with mixtures of oligonucleotides [typically a mixture of octomers of all possible sequences] that are each immobilized on a matrix with memory that is encoded with the sequence of the probe. Upon hybridization, hybridizing probes are identified by routine methods, such as OD or using labeled probe, and the sequences of the hybridizing probes can be determined by retrieving the sequences from the linked memories. When hybridization is carried out under conditions in which no mismatches are tolerated, the sequence of the target can then be determined by aligning overlapping sequences of the hybridizing probes.

Previous methods used to accomplish this process have incorporated microscopic arrays of nucleotide oligomers synthesized on small silicon based chips. It is difficult to synthesize such arrays and quality control the large number of spots on each chip [about 64,000 spots for 8-mer oligonucleotides, that number necessary to accomplish sequencing by hybridization].

In the present method, each oligomer is independently synthesized on a batch of individual chips, those chips are tested for accuracy and purity of their respective oligomers, then one chip from each batch is added to a large pool containing oligomers having all possible sequences. After hybridization in batch mode with the gene segment to be sequenced, usually amplified by a method such as PCR, using appropriate primers, and labeled with a detectable [such as fluorescent] tag, the chips can be passed through a detector, such as described above for processing multiplexed assays,including multiplexed immunoassays, and the degree of binding to each oligomer can be determined. After exposing the batch to varying degrees of dissociating conditions, the devices can again be assayed for degree of binding, and the strength of binding to related sequences will relate the sequence of the gene segment [see, e.g., International PCT Application WO 95/00530].

An exemplary method for synthesizing an oligonucleotide library, preferably hexamers or octomers, for use in sequencing methods, or other methods, is set forth in the EXAMPLES and depicted in FIG. **33**, in which the oligonucleotides are synthesized on optical memory devices that are each uniquely encoded either before, during or after synthesis with a code. The identity of the oligomer associated with each code is stored in a remote memory, generally a computer. Other memory with matrices, such as the MICROTUBES™ and MICROKANS™ and other such combination, may be used in place of the optical memory devices

In particular this library of microreactors [oligonucleotides-linked to memory with matrix] can be used in methods for DNA sequencing by primer walking using capillary electrophoresis (CE) and ultrathin slab gels for separation [see, e.g., Ruiz-Martinez et al. (1996) *Biotechniques* 20:1058–1069; Kieleczawa et al. (1992) *Science* 258:1787–1791; McCombie et al. (1994) *Biotechniques* 17:574–5790]. Such methods rely on the use of oligonucleotide libraries, containing all permutations of pentamer or hexamers, which are used as primers. The identity of each oligonucleotide will be encoded in the associated memory or stored in the proximate or linked memory. A synthetic protocol is depicted in FIG. **33**.

i. Separations, Physical Mapping and Measurements of Kinetics of Binding and Binding Affinities

Multiple blots [i.e., Western, Northern, Southern and/or dot blots] may be simultaneously reacted and processed. Each memory, in the form of a rectangle or other suitable, is linked or coated on one surface with material, such as nitrocellulose, to which or the analyte of interest binds or with which it reacts. The chips are arranged in an array, such as in strips that can be formed into rectangles or suitable other shapes, circles, or in other geometries, and the respective x-y coordinate or other position-identifying coordinates, and, if needed, sheet number and/or other identifying information, is programmed into each memory. Alternatively, they may be programmed with this identification, then positioned robotically or manually into an array configuration. They are preferably linked together, such as by reversible glue, or placing them in agarose, or by any suitable method as long as the reactive surface is not disturbed. Following transfer of the material, such as transfer of protein from a Western Blot, nucleic acid from a Southern or Northern blot, dot blots, replica plated bacterial culture, or viral plaques, the memories are separated and mixed for reaction with a traditionally labeled, such as a fluorescent label, detection nucleic acid, protein, antibody or receptor of interest. Complexes are identified, and their origin in the blot determined by retrieving the stored information in each chip. Quantitation may also be effected based on the amount of label bound.

A series of appropriately activated matrices with memories are arranged in an array, one or, preferably two dimensional. In one configuration, each chip is pre-programmed and placed in a specific location that is entered into its memory, such as an x-y coordinate. At least one surface of the memory with matrix is treated so that the transferred reagent binds. For example, a piece of nitrocellulose can be fixed to one side of the memory device. The resulting array is then contacted with a separation medium whereby each reagent of interest is transferred to and bound to the end of the matrix with memory such that the reagent location is known. The matrices are separated and pooled; multiple arrays may be pooled as long as source information is recorded in each memory. All matrices with memories are then contacted with detection agents that specifically bind to reagents in the mixture. The matrices with memories are passed through a reading device, either after an incubation for end point determinations or continuously for kinetic measurements. The reading devices is a device that can detect label, such as fluorescence, and an reader, such as an RF ready, that can query the memory and identify each matrix. The rate of binding and maximum binding and identify of bound reagents can be determined. Dot blots, for example, can be used in hybridoma analysis to identify clones that secrete antibodies of desired reactivity and to determine the relative affinities of antibodies secreted by different cell lines. Matrices with memories that are activated to bind immunoglobulins and with on-board information specifying their relative locations in the array are dipped in an array into the wells of microplates containing hybridoma cells. After incubation, they are withdrawn, rinsed, removed and exposed to labeled antigen. Matrices of desired specificity and affinity are selected and read thereby identi-

fying the original wells containing the hybridoma cells that produce the selected antibodies.

In other embodiments, the transfer medium [i.e., the nitrocellulose or other such medium] may be part of the surface of the chip or array of chips that can bind to the separated species subsequent to separation. For example, the separation system, such as the agarose or polyacrylamide gel, can be included on the surface(s) of the matrix with memories in the array. After separation the surface will be activated with a photoactivatable linker or suitable activating agent to thereby covalently link, such as by a photoflash, the separated molecules to the matrices in the array.

Alternatively, each matrix with memory may have one or more specific binding agents, such as an antibody or nucleic acid probe, attached (adsorbed, absorbed, or otherwise in physical contact) to matrix with memory. The matrix with memory and linked binding agent is then contacted with a medium containing the target(s). After contacting, which permits binding of any targets to which the linked binding agents specifically bind, the matrix with memory is processed to identify memories with matrices to which target has specifically bound via interaction with the binding agent. For example, the (1) the target is labeled, thereby permitted direct detection of complexes; (2) the memory with matrix is then contacted with a developing agent, such as a second antibody or detection probe, whereby binding agent-target complexes are detected; or (3) the detection agent is present during the reaction, such as non-specifically attached to the matrix with memory or by other method [thin film, coated on the matrix with memory, coated on nitrocellulose].

Such support bound analytes may also be used to analyze the kinetics of binding by continuously passing the supports through a label reading device during the reaction, and identify the labeled complexes. The binding agents can be eluted, either in a kinetically readable manner or in batch. In addition, since the recording devices may also include components that record reaction conditions, such as temperature and pH, kinetics, which are temperature and pH dependent, may be accurately calculated.

After elution, the support bound analytes may be identified to analyze kinetics of binding to the binding agent. Such binding and elution protocols may also be adapted to affinity purification methodologies.

j. Cell Sorting

The devices herein may also be used in methods of cell sorting. For example, the memory with matrix combinations are linked to selected antigens, information regarding the antigens is encoded into the memories, the resulting combinations are used in multi-analyte analyses of cells.

It is possible to identify a profile of cells exhibiting different surface markers [antigens, for example, or other ligands or receptor molecules] by using combinations of labeled and matrix memory-bound binding agents. In one embodiment, each agent, such as an antibody, capable of binding specifically to one of many different surface markers is bound to a different matrix with a memory. The nature of the recognized marker is recorded in the memory of each matrix-binding agent complex, and the mixture of binding-agent-matrix memory complexes is reacted with a mixture of cells. The cell-matrix complexes that result from binding agents attaching cells to the surfaces of the respective matrices are then reacted with a labeled [for example, fluorescent] reagent or mixture of reagents which also reacts with the cells. These labeled reagents can be the same or different from those coupled to the memory matrices. When the matrices are passed through a reader [to read the label and the memory], those that have bound cells can be identified and if necessary isolated. This application is particularly useful for screening for rare cells, for example stem cells in a bone marrow or peripheral lymphocyte sample, for detecting tumor cells in a bone marrow sample to be used for autologous transplantation, or for fetal cells in a maternal circulation.

In these embodiments, the memory with matrices herein can be counted and read with instruments, such as a device that operates on the principles of a Coulter counter, that are designed to count cells or particles. In using a Coulter Counter, a suspension of cells or particles is sucked through a minute hole in a glass tube. One electrode is placed within the tube and another is outside of the tube in the suspension. The passage of a particle through the hole temporarily interrupts the current; the number of interruptions is determined by a conventional scaling unit.

For use herein, such instruments are modified by including an RF reader [or other reader if another frequency or memory means is selected] so that the identity of the particle or cell [or antigen on the cell or other encoded information) can be determined as the particle or cell passes through the hole and interrupts the current, and also, if needed, a means to detect label, such as fluorescent label. As the particle passes through the hole the RF reader will read the memory in the matrix that is linked to the particle. The particles also may be counted concurrently with the determination of the identity of the particle. Among the applications of this device and method, is a means to sort multiple types of cells at once.

k. Multiplexed or Coupled Protocols in Which the Synthesis Steps [the Chemist] is Coupled to Subsequent Uses of the Synthesized Molecules

Multiplexed or multiple step processes in which compounds are synthesized and then assayed without any intermediate identification steps are provided herein. Since the memories with matrices permit identification of linked or proximate or associated molecules or biological particles, there is no need to identify such molecules or biological particles during any preparative and subsequent assaying steps or processing steps. Thus, the chemistry [synthesis] can be directly coupled to the biology [assaying, screening or any other application disclosed herein]. For purposes herein this coupling is referred to as multiplexing. Thus, high speed synthesis can be coupled to high throughput screening protocols.

H. Applications of the Memories With Matrices and Luminescing Matrices With Memories in Combinatorial Syntheses and Preparation of Libraries

Libraries of diverse molecules are critical for identification of new pharmaceuticals. A diversity library has three components: solid support matrix, linker and synthetic target. The support is a matrix material as described herein that is stable to a wide range of reaction conditions and solvents; the linker is selectively cleavable and does not leave a functionalized appendage on the synthetic target; and the target is synthesized in high yield and purity. For use herein, the diversity library further includes a memory or recording device in combination with the support matrix. The memory is linked, encased, in proximity with or otherwise associate with each matrix particle, whereby the identify of synthesized targets is written into the memory.

The matrices with memories are linked to molecules and particles that are components of libraries to electronically tagged combinatorial libraries.

Particularly preferred libraries are the combinatorial libraries that containing matrices with memories that employ radio frequencies for reading and writing.

1. Oligomer and Polypeptide Libraries

a. Bio-oligomer Libraries

One exemplary method for generating a library [see, U.S. Pat. No. 5,382,5131 involves repeating the steps of (1) providing at least two aliquots of a solid phase support; separately introducing a set of subunits to the aliquots of the solid phase support; completely coupling the subunit to substantially all sites of the solid phase support to form a solid phase support/new subunit combination, assessing the completeness of coupling and if necessary, forcing the reaction to completeness; thoroughly mixing the aliquots of solid phase support/new subunit combination; and, after repeating the foregoing steps the desired number of times, removing protecting groups such that the bio-oligomer remains linked to the solid phase support. In one embodiment, the subunit may be an amino acid, and the bio-oligomer may be a peptide. In another embodiment, the subunit may be a nucleoside and the bio-oligomer may be an oligonucleotide. In a further embodiment, the nucleoside is deoxyribonucleic acid; in yet another embodiment, the nucleoside is ribonucleic acid. In a further embodiment, the subunit may be an amino acid, oligosaccharide, oligoglycosides or a nucleoside, and the bio-oligomer may be a peptide-oligonucleo-tide chimera or other chimera. Each solid phase support is attached to a single bio-oligomer species and all possible combinations of monomer [or multimers in certain embodiments] subunits of which the bio-oligomers are composed are included in the collection.

In practicing this method herein, the support matrix has a recording device with programmable memory, encased, linked or otherwise attached to the matrix material, and at each step in the synthesis the support matrix to which the nascent polymer is attached is programmed to record the identity of the subunit that is added. At the completion of synthesis of each biopolymer, the resulting biopolymers linked to the supports are mixed.

After mixing an acceptor molecule or substrate molecule of interest is added. The acceptor molecule is one that recognizes and binds to one or more solid phase matrices with memory/bio-oligomer species within the mixture or the substrate molecule will undergo a chemical reaction catalyzed by one or more solid phase matrix with memory/bio-oligomer species within the library.

The resulting combinations that bind to the acceptor molecule or catalyze reaction are selected. The memory in the matrix-memory combination is read and the identity of the active bio-oligomer species is determined.

b. Split Bead Sequential Syntheses

Various schemes for split bead syntheses of polymers [FIG. 1], peptides [FIG. 2], nucleic acids [FIG. 3] and organic molecules based on a pharmacophore monomer [FIG. 4] are provided. Selected matrices with memory particles are placed in a suitable separation system, such as a funnel [see, FIG. 5]. After each synthetic step, each particle is scanned [i.e., read] as it passes the RF transmitter, and information identifying the added component or class of components is stored in memory. For each type of synthesis a code can be programmed [i.e., a 1 at position 1,1 in the memory could, for example, represent alanine at the first position in the peptide]. A host computer or decoder/encoder is programmed to send the appropriate signal to a transmitter that results in the appropriate information stored in the memory [i.e, for alanine as amino acid 1, a 1 stored at position 1,1]. When read, the host computer or decoder/encoder can interpret the signal read from and transmitted from the memory.

In an exemplary embodiment, a selected number of beads [i.e., particulate matrices with memories [matrix particles linked to recording devices], typically at least 10^3, more often 10^4, and desirably at least 10^5 or more up to and perhaps exceeding 10^{15}, are selected or prepared. The beads are then divided into groups, depending upon the number of choices for the first component of the molecule. They are divided into a number of containers equal to or less than [for pooled screening, nested libraries or the other such methods] the number of choices. The containers can be microtiter wells, Merrifield synthesis vessels, columns, test tubes, gels, etc. The appropriate reagents and monomer are added to each container and the beads in the first container are scanned with electromagnetic with radiation, preferably high frequency radio waves, to transmit information and encode the memory to identify the first monomer. The beads in the second container are so treated. The beads are then combined and separated according to the combinatorial protocol, and at each stage of added monomer each separate group is labeled by inputting data specific to the monomer. At the end of the synthesis protocol each bead has an oligomer attached and information identifying the oligomer stored in memory in a form that can be retrieved and decoded to reveal the identity of each oligomer.

An 8-member decapeptide library was designed, synthesized, and screened against an antibody specifically generated against one of the library members using the matrices with memories. Rapid and clean encoding and decoding of structural information using radio frequency signals, coupling of combinatorial chemical synthesis to biological assay protocols, and potential to sense and measure biodata using suitable biosensors, such as a temperature thermistor or pH electrode, embedded within the devices have been demonstrated. The "split and pool" method [see, e.g., Furka et al. (19910 *Int. J. Pent. Protein Res.* 37:487–493; Lam et al. (1991) *Nature* 354:82–84; and Sebestyén et al. (1993) *Bioorg. Med. Chem. Lett.* 3:413–418] was used to generate the library. An ELISA [see e.g., Harlow et al. (1988) *Antibodies, a laboratory manual*, Cold Spring Harbor, N.Y.] was used to screen the library for the peptide specific for the antibody.

2. "Nested" Combinatorial Library Protocols

In this type of protocol libraries of sublibraries are screened, and a sublibrary selected for further screening [see, e.g., Zuckermann et al. (1994) *J. Med. Chem.* 37:2678–2685; and Zuckermann et al. (1992) *J. Am. Chem. Soc.* 114:10646–10647]. In this method, three sets of monomers were chosen from commercially available monomers, a set of four aromatic hydrophobic monomers, a set of three hydroxylic monomers, a set of seventeen diverse monomers, and three N-termini were selected. The selection was based on an analysis of the target receptor and known ligands. A library containing eighteen mixtures, generated from the six permutations of the three monomer sets, times three N-termini was prepared. Each mixture of all combinations of the three sets of amines, four sets of hydrophobic monomers and seventeen diverse monomers was then assayed.

The most potent mixture was selected for deconvolution by synthesis of pools of combinatorial mixtures of the components of the selected pool. This process was repeated, until individual compounds were selected.

Tagging the mixtures with the matrices with memories will greatly simplify the above protocol. instead of screening each mixture separately, each matrix particle with memory will be prepared with sets of the compounds, analogous to the mixtures of compounds. The resulting matrix particles with memories and linked compounds can be combined and then assayed. As with any of the methods provided herein, the linked compounds [molecules or biological particles] can be cleaved from the matrix with memory prior to assaying or anytime thereafter, as long as the cleaved molecules remain in proximity to the device or in some manner can be identified as the molecules or particles that were linked to the device. The matrix particle(s) with memories that exhibit the highest affinity [bind the greatest amount of sample at equilibrium] are selected and identified by querying the memory to identify the group of compounds. This group of compounds is then deconvoluted and further screened by repeating this process, on or off the matrices with memories, until high affinity compounds are selected.

3. Other Combinatorial Protocols

The matrices with memories provided herein may be used as supports in any synthetic scheme and for any protocol, including protocols for synthesis of solid state materials. Combinatorial approaches have been developed for parallel synthesis of libraries of solid state materials [see, e.g., Xiang et al. (1995) *Science* 268:1738–1740]. In particular, arrays containing different combinations, stoichiometries, and deposition sequences of inorganics, such as $BaCO_3$, BiO_3, CaO, CuO, PbO, $SrCO_3$ and Y_2O_3, for screening as superconductors have been prepared. These arrays may be combined with memories that identify position and the array and/or deposited material.

I. Microvessel Opening and Closing Devices

In order to facilitate the opening and closing of a MICROKAN microvessel or other such microvessel or microreactor, a pair of hand tools is provided herein. More specifically, and with reference to U.S. Pat. Nos. 4,651,598 and 4,662,252, each of the hand tools includes a pliers body that has been adapted to accept various portions of the microvessel. Referring to FIG. **44**, the cap sealing tool is shown and generally designated **4600**. As shown, the tool **4600** includes a pair of elongated handle portions **4602** and **4604**. These handle portions articulate about a sliding pivot disc **4605** that engages any one of the teeth **4607** such that when the handles are forced together, the opposite ends of the handle portions also move towards each other. On the ends of the elongated handle members, the traditional pliers are modified to have a striking plate **4610** and a receiving cylinder. The receiving cylinder is pivotally attached to the end of member **4602** such that the cylinder may swing out away from the pliers, to facilitate loading and unloading the cylinder **4608**. On the upper member **4612**, the striking plate **4610** is attached to be positioned directly above the cylinder when the cylinder is in its raised position. FIG. **45** is a front cross-sectional view showing the placement of the microvessel within the cylinder such that the lid **4620** is positioned over the tube **4616**. Referring to FIG. **46**, the striking plate **4610** and cylinder **4622** are shown in lateral cross-section. From this view it is clear that the inside of the cylinder **4622** is formed to accept a MICROKAN microvessel. As can be appreciated, however, the cylinder may be formed to accept virtually a container of any geometry or size, preferably a container with a volume of 1 ml or less, that is sealed as provided herein. The MICROKAN microvessel shown here contains a tag **4618**, such as, for exemplification purposes, an RF tag in the shape of a capsule, such as that available from IDTag, described elsewhere herein. As with the microvessel, the tags may be formed in a variety of shapes and sizes, as long as it is insertable into the container. FIG. **45** shows the lid **4620** of the tube **4616** positioned above the tube such that when the handle portions are squeezed together, the lid **4620** is forced into the container to seal the tag therein.

Moving now to FIG. **47**, the cylinder **4622** is shown forced against the striking plate **4610** to press the lid **4614** into the microvessel **4616**. Once the tag is contained within the microvessel, the pliers are opened and the cylinder is articulated outwards about the pinion **4626** in direction **4628** such that the tang **4626** strikes the bottom **4630** of the microvessel **4616** to push the microvessel out of the cylinder. Once the cylinder is articulated, the microvessel may be easily removed from the cylinder. As can be seen from this view, the tag **4618** is sealed inside the microvessel to prevent exposure of the tag to environmental contaminates. Moreover, by placing the tag in a sealable container, the tag may be reused.

Because the tag **4618** may be reused, another tool has been created to facilitate removing it from the microvessel. Referring now to FIG. **49**, a similar plier-like tool is formed with a striking plate **4610**, and a fork **4634**. The fork is attached to the end of elongated handle **4604** and has two prongs **4632** which are connected to define an arc shaped wedge. This arc shaped wedge can be positioned against the side of the microvessel where the lid **4614** joins the microvessel, and upon actuating the pliers, the prongs slide between the lid and the microvessel to remove the lid.

As perhaps more clearly shown in FIG. **50**, the prongs **4632** are wedge shaped such that the more the pliers are closed, the more the lid is urged out of the microvessel. From this view, the shape of the striking plate **4610** can be easily seen. It is to be appreciated that any number of striking plate shapes could be used. In fact, because a container of any shape could be used to hold the tag, it should be appreciated that the striking plate could be shaped to accommodate those shapes. Further, the prongs **4632** on the fork **4634** could be shaped to more closely fit an alternatively shaped container. Once the lid is removed, the tag can also be removed from the microvessel and reused in another microvessel.

J. Sleeves With Memories

In another embodiment provided herein, depicted in FIGS. **35–43**, a sleeve, containing a remotely programmable memory with a coiled antenna, that is specially adapted to fit on a tube, such as an Hewlett Packard HPLC tube. The sleeve fits tightly on the tube, thereby permitting the tube to be tracked and information about the contents, source or other some information, to be stored in the memory in the sleeve or in a remote computer that associates the memory with such information.

Referring to FIG. **35**, the identification system is shown and generally designated **3000**. The system **3000** includes a read/write controller **3002** that includes a housing **3018** that supports a carousel **3006**, and a computer system **3004**. The carousel **3006** as shown is mounted to rest on the top surface

3020 of the housing 3018. As will be further discussed below in connection with FIG. 36, the carousel rotates about its vertical axis so that as it rotates, each of the ports 3008 will rotate in front of the plunger 3024. The carousel is formed with a number of the ports 3008 that are sized to receive a vial 3010. It is to be appreciated that although the carousel is shown to contain 21 different ports, a carousel having any number of ports could be formed.

Formed in the carousel is a keyway 3012 that is sized to accept a key 3014. While not shown in this Figure, this key is attached to a hub 3056 (shown in FIG. 37). In an effort to stop the carousel from freely rotating, the plunger 3024 is positioned on the top surface 3020 so that its slide 3044 (shown in FIG. 36) strikes the outside rim of the carousel.

The computer system 3004 includes a central processing unit 3030 that has a serial port to accept a serial cable 3038, a monitor 3032 having a screen 3034, and a keyboard 3036. While a traditional computer is shown to include separate parts, a laptop computer would be equally acceptable so long as it is equipped with a serial port to accept cable 3038.

Referring now to FIG. 36, the read/write controller is shown from the top thereby providing a more detailed view of the rotational positioning of the carousel 3006 and the plunger 3024. The plunger is attached to the top surface 3020 of the housing 3018 with a flange 3042. The positioning of the plunger is important because the plunger slide 3044 must hit the outer rim of the carousel in a location that will provide a stopping pressure against the carousel., This stopping pressure acts to prevent any spinning of the carousel unless there is a turning force placed on the carousel itself. It will be appreciated that the strike of the plunger could be replaced by a more precise stopping member, such as a post. The post, like the slide, would strike the outer surface of the carousel. If the post is significantly smaller than the slide, the carousel may be formed with a number of holes spaced along the rim of the carousel so that when activated, the plunger would urge the post into the hole to securely stop the carousel from spinning. In addition, any other method of orienting the carousel on the housing could be used, so long as the carousel could be rotated.

The surface 3016 of housing 3018 is attached using screws 3022.

Instead of using screws, virtually any manner of attaching the housing together may be used. The housing 3018 may be formed from one single piece of material that is either bored or machined to have a hollow inside for holding the necessary electronics discussed below.

Also from FIG. 36, the interaction between the key 3014 and keyway 3012 is clearly shown. From this view, it is to be appreciated that the carousel could simply be lifted off of the housing and keyway and replaced with another carousel that was oriented such that the keyway in the new carousel would match the position of the key on the housing. In such a manner, virtually any number of carousals could be placed on the housing.

The outer surface of the carousel is formed with 21 ports 3008. Each of these ports is formed with a cutout 3009. As easily appreciated from this view, the carousel could be made to have a larger diameter to accommodate a larger number of vials, or the vials could be smaller to accomplish the same quantity of vials. In other words, by making the carousel and vials of differing sizes, a virtually unlimited number of vials could be accommodated. Moreover, because the carousals are removable, a large number of vials could be processed through the read/write controller in a short period of time. It should also be appreciated that although the carousel is formed with a handle 3026, it could instead be formed with a grip that would be easily mated with some automated handling device, such as a robotic arm (not shown). This robotic arm could be independently operated, or could be controlled by the computer system 3004.

There is a notch 3040 formed in the outer edge of the carousel that is particularly useful for aligning with the plunger 3024 when installing or removing the carousel from the read/write controller. As is to be appreciated, when the carousel is rotated such that the notch is adjacent the plunger 3024, the slide 3044 will not strike the carousel. As a result, the carousel may be easily lifted up and off of the read/write controller. Such simple removal is particularly useful when there are a large number of vials present on the carousel that would add a significant weight to the carousel, making removal more strenuous.

Referring now to FIG. 37, the housing 3018, carousel 3006, vial 3010, and plunger 3024 are shown in cross-section. The housing is shown having a module 3068 that includes the majority of the electronics needed to operate the system 3000, with the exception of the computer system 3004. Mounted and extending through the housing 3018 is a motor 3052. The motor is positioned to extend vertically upwards from the housing and into a hub 3056 that is sized to receive the carousel 3006. Specifically, the shaft 3054 of the motor extends upwards and is formed with a locking tab 3058 that is intended to keep the hub properly positioned on the shaft. In other words, the tab 3058 engages the hub to prevent the hub from rotating when the shaft 3054 is not rotating. To assist the shaft in maintaining a proper vertical alignment, a bearing 3082 is mounted to the housing 3018. Any type of bearing could be used for this application, including but not limited to ball bearings or roller bearings. In fact, a grease bearing, if providing sufficient stability, could be used. In the event that the carousel is to be manually rotated, there would be no need for the motor 3052 and, as a result, the bearing 3082 would be of a different type to give support to the carousel itself. In such an instance, the shaft would be fixed to the housing, or to a bearing mounted on the housing, and the carousel would rotate freely about the shaft. Thus, in such an instance where no motor is used, the tabs 3058 would not be formed on the shaft 3054.

In order to control the motor 3052, if used, a control wire 3074 extends from the motor to the module 3068. One function of this module would be to receive an electronic signal from the computer system and command the motor to a particular rotational position. In order to achieve this positional accuracy, the motor may be a stepper motor, or may be equipped with either a synchro or resolver that would be used to determine the angular position of the carousel 3006. Briefly, and as is generally known in the art, a synchro or resolver would provide a three-phase electrical signal that would represent the angular position of the carousel. This three-phase signal can be decoded using a synchro or resolver-to-digital converter to determine the angular position of the carousel in digital representation. Alternatively, the carousel could be formed with angular markings on the outer surface of the carousel that could be read by either a mechanical or optical device common for use in such positioning systems. Such an optical system could use a decoding scheme based on a binary-coded-decimal representation in a bar-code form that could be easily marked on the outer surface of the carousel.

Plunger 3024 is shown with slide 3044 striking the outer surface of vial 3010 to hold the rotational position of the carousel. As shown, the plunger 3024 has a slide 3044 that is urged towards the carousel by a spring 3046. As a result

of the expansion of the spring, the slide is urged gently against the surface of the carousel. Thus, in order to adjust the force with that the slide strikes the carousel, the spring **3046** may be selected to have a different spring constant. In other words, the higher the spring constant, the more force the slide will strike the carousel with. Alternatively, the plunger could be formed with slots that would allow the screws **3043** to be loosened to adjust the plunger position either towards or away from the carousel to effectively adjust the force with which the slide strikes the carousel. The spring **3046** is retained in place within the plunger **3024** by the combination of nipple **3066** and nipple **3064**. The diameter of the nipples is to be selected to match the diameter of the spring. As such, the diameter of the spring and nipples will likely change to reflect springs having different spring constants and dimensions.

In an alternative embodiment, the spring **3046** could be substituted with a solenoid that could be electrically activatable to force the slide either towards or away from the carousel. Such electrical control could easily come from the module **3068**, in combination, or acting independently, with computer **3004**. Also, if the slide is replaced with the post as mentioned above, the accuracy of the positioning could be increased while not increasing the level of human intervention required to position the carousel on the housing.

A vial **3010** is shown placed in a port **3008** of the carousel, and aligned with a receiving coil **3062**. Importantly, the slide **3024** holds the vial **3010** directly over the antenna coil **3062**. While this is not particularly necessary to insure proper communication between the vial and the antenna coil, the need for accuracy of the positioning increases when the distance between the vials is decreased. As discussed above, where there is a large number of vials held on a single carousel, there is a need to properly position the vial so that there is limited, or no, interference between the intended vial and any neighboring vials. This problem would be particularly noticeable in carousals where there is minimal space between the vials, or when the vials have been miniaturized.

Referring now to FIG. **38**, a vial is shown in perspective and generally designated **3010**. Vial **3010** has three portions: a lid **3076**, a cylinder **3028**, and a sleeve. The vial is shown in cross-section in FIG. **39**. As shown, the lid **3076** is held on the cylinder **3028** by threads **3090** that are formed on the cylinder and the lid. Although threads provide for an easy installation and removal of the lid, a snap-type attachment could also be used. Moreover, any type of lid could be used and it would not affect the utility of this system **3000**. It will be appreciated by those skilled in the art that the ability to add and remove materials from the cylinder is determined by the type of lid that is used. This preferred embodiment uses a lid **3076** that is formed with a membrane window **3078** that is preferably made of a puncturable material. This puncturable material would allow a syringe to puncture the membrane to inject or remove materials from the cylinder, removing the syringe when finished, allowing the membrane to re-seal itself. Such materials are well known in the art and are not discussed further here.

The cylinder **3028** is shown filled with material **3050**. While this is shown as a fluid, it is to be appreciated that any number of materials, such as polystyrene beads, patient samples and other materials, could be placed in these cylinders. A sleeve **3048** is attached to the bottom of the cylinder **3028** and is formed with an upper orifice **3116** and a lower chamber **3117**. The sleeve **3048** is shown in greater detail in FIG. **40**. The upper orifice **3116** is formed with a circumferential ridge **3110** that is adjacent the upper end of the sleeve. This ridge **3110** is sized to have a distance **3118** between them that is slightly smaller than the diameter of the cylinder **3028**. This diameter difference is important to insure that the sleeve, once positioned, will not slide off of the cylinder. This is particularly important when it is critical to track the placement and location of a vial. In order to achieve the resilience needed to allow the insertion of the cylinder into the sleeve. The sleeve is fabricated from a suitable insert material, such as polypropelyne material. While this material is fairly pliable, it is also sufficiently rigid to retain its shape. As a result, the polypropylene is particularly suited to such an application where there is a need to securely mount the sleeve to an object, while providing sufficient pliability to avoid cracking the cylinder if made of a fragile material such as glass. In situations where the cylinder is more rigid and less fragile, the sleeve could be attached using a variety of other manners. More specifically, elastic bands or i-adhesive straps could be used to hold the sleeve in position over the cylinder.

In order to facilitate the insertion of the cylinder **3028** into the orifice **3116** in the sleeve **3048**, a vent hole **3019** is formed in the wall of the sleeve adjacent the divider **3114** of the orifice **3116**. This vent hole allows the air trapped inside the orifice while the cylinder is being inserted to escape into the atmosphere. It is to be appreciated that if there were no vent hole **3019** formed in the sleeve, a considerable pressure would build within the orifice thereby preventing the insertion of the cylinder.

On the underside of the sleeve, a chamber is formed between the plug **3092**, sleeve wall **3112**, and divider **3114**. Within this chamber is the sleeve antenna **3090**, a microchip **3094**, both mounted on a substrate **3096**. The sleeve antenna **3090** includes multiple windings of a fine gauge, insulated wire to form an inductive proximity antenna. The antenna is an important feature of the data transmission process between the read/write controller and read/write device. The antenna in this embodiment has a outer coil diameter of 0.420 inches and an inner coil diameter of 0.260 inches. The coils includes approximately **305** turns of wire having a diameter of 0.015 inches. In any case, the inductance of the antenna once formed should be on the order of 7.92 mH, with a series resistance of no more than 850 ohms at 1 volt and 100 KHz. Coil antennas of differing sizes can be used in this embodiment, but the number of turns of wire will change with the size of the coil, the size of the wire, and the overall diameter of the coil. To determine the proper number of turns given a different dimensioned antenna, the following equation must be used:

$$L = 2 * a * \ln\left(\frac{a}{D} - K\right) * N^{1.9}$$

where "L" is the desired inductance in nH, "a" is the antenna circumference in centimeters, "D" is the wire diameter in centimeters, "N" is the number of windings, "K" is the geometrical constant that for a circular antenna is 1.01, and for a square antenna is 1.47. Because the value of "K" for a circular antenna is approximately and is normally much smaller than a/D, it can be left out, yielding a simpler equation:

$$N \approx 1.9\sqrt{\frac{L}{2 * a * \ln(a/D)}}$$

Thus, once the inductance desired, diameter of the wire and circumference of the antenna are known, the proper number of windings can be determined. Or conversely, any one

variable can be determined from the equations above to yield the characteristics of the antenna.

In this particular embodiment, the cylinders are vials fabricated from glass. It is to be appreciated, however, that the cylinder could be made of virtually any material, including metal or any other inert material. They may also be made of matrices that include at least a portion of a surface suitable for linking biological particles or molecules. In the event metal is used, there may be a need to elongate the sleeve to insure that the metal is distanced from the antenna. In addition to the cylinder being made of a variety of materials, it should also be appreciated that any the sleeve can be used with any number of containment devices. Of these devices, the discussed above would be a particularly well suited container for attachment to the sleeve. In fact, the sleeve could be reformed to a substantially different structure that, nonetheless, would work equally as well as the sleeve and cylinder embodiment. Moreover, any other containment device or matrix support that is provided herein is capable of being equipped with sleeve with memory. It is to be appreciated, however, that particularly small-sized read/write device's would require a miniaturized design having a smaller sleeve antenna. In fact, in environments where a sleeve is not practical, the read/write device may be made in the form of a submersible chamber or embedded in the structure of the containment device as described herein.

Also within the chamber **3117** is the microcircuit **3094** and substrate **3096**. The microcircuit includes a rectifier, voltage regulator, reset generator, demodulator, clock extractor, modulator, control unit, and memory [see, U.S. Pat. No. 5,345,231]. Referring briefly to FIG. **2** in the U.S. Pat. No. 5,345,231 patent, the overall system architecture of the semiconductor is identified. This circuitry derives its power from rectifying an incoming signal that is received by the antenna. The rectified signal is conditioned with the voltage regulator and fed to all other circuitry on the semiconductor. The microcircuit **3094** is attached to the substrate **3096** using a bonding adhesive as is common in the art. Any number of attachment methods could be used, however, and would achieve the same result. The substrate is preferably made of alumina that would provide a stable platform that would have dielectric constants approximately equal to those of the silicon wafer of the microcircuit. As is common in the industry, by matching the dielectric constants of the materials, as well as the thermal expansion coefficients of the various materials, there is less of a likelihood that there will be cracking on the microcircuit.

Moreover, because the thermal coefficients of the microcircuit are approximately the same as the alumina substrate, there is a good match between the two that greatly reduces the thermal stresses that are present on the microcircuit.

Referring now to FIG. **41**, the substrate **3096** is shown with the microcircuit **3094** firmly attached. In addition to having the microcircuit mounted to it, the substrate **3096** is also formed with a copper layer that is intended to be the bonding points for the antenna leads **3102**. More specifically, the substrate **3096** is formed with a pair of pads **3100**. Each of these pads is securely attached to the surface of the substrate to allow the wires **3098** from the microcircuit, called bonding wires, to be easily attached to the antenna wires **3102**. Attachment between the two wires is made by soldering or brazing one of the wires, preferably the most delicate wire that would be the bonding wire **3102**, from the microcircuit to the pad **3100**. Once the most delicate wire is attached, the more robust and durable wire is attached to the pad. In this instance, the antenna wire is significantly thicker than the bonding wire and, thus, is connected last. In order to simplify the attachment of the antenna wire and to reduce the heat to which the microcircuit is exposed to, the pad may be tinned. A lead is "tinned" when covered with a slight layer of solder to which the antenna wire can be more easily attached to.

Once the sleeve antenna **3090** is formed and electrically connected to the substrate **3096**, the substrate is attached to the antenna using an adhesive **3097**. This adhesive may be of any kind known in the art and is selected so that it produces very little radio frequency for other frequency when other frequencies are used] interference. In this embodiment, silicone is used to secure the substrate to the sleeve antenna **3090**. It is important to note that the microcircuit **3094** in the present embodiment fits nicely within the internal diameter of the sleeve antenna. This is of particular usefulness because such positioning effectively reduces the height of the combination of the antenna, substrate, and microcircuit. Once the substrate is firmly attached to the sleeve antenna, the combination is inserted into the chamber **3117** and held against the divider **3114** by an adhesive. Again, silicone is used to hold the sleeve antenna in place against the divider. As shown in FIG. **40**, there is little space remaining following the insertion of the sleeve antenna and substrate into the chamber.

Once the sleeve antenna is positioned within the chamber, the plug **3092** is inserted into the sleeve **3048** to seal the chamber **3117**. The plug is formed with a circumferential ring **3124** which engages to a circumferential slot **3122** formed in the sleeve wall. The ring and slot engage each other to insure that the plug does not fall out, thereby exposing the delicate electronics to the environment. In an effort to further reduce the likelihood of exposure to the environment, particularly when the environment is damaging to the electronics (e.g. wet), the plug can be sealed in place against the sleeve using a heated tool. Referring to FIG. **42**, a heating tool is shown that contains a thermally conducting block **3134** that is formed with a hole **3132** for receiving the sleeve. Specifically, once the plug has been inserted into the sleeve to seal the chamber, the entire device is inserted into the block **3134** that has been previously, or currently, heated. Because, as mentioned above, the sleeve is preferably made out of polyethylene, it is easily melted. Thus, by exposing the plug end of the sleeve to a heat sufficient to barely melt it, the plug is fused to the sleeve to create an environment seal. This seal is capable of withstanding even the most harsh environment, while maintaining the electronics in a dry location within the chamber.

Referring back to FIG. **37**, and having review all necessary structural components of the system **3000**, the operation of the system is more easily understood. A vial is either filled with a substance, or has been purchased with a substance already in it. In either case, this vial is inserted into a sleeve. As the sleeve is inserted over the vial, the polyethylene yields to provide a solid connection between the sleeve and the cylinder wall **3028**. Preferably, at the time of mating the cylinder with the sleeve, the microcircuit has no memory stored on it which represents an identification tag. Thus, once the vial and sleeve have been combined, the combination is placed in a port **3008** of the carousel **3006**. Once inserted in the port, the carousel is then placed on the housing with the notch **3040** adjacent the plunger **3024**. Then, the carousel is rotated such that the vial is directly in front of the plunger, and thus, immediately over the base antenna **3062**. Once in position, the base antenna **3062** is excited with a high frequency (HF) signal from the base antenna **3062** [see, U.S. Pat. No. 5,345,231], which thereby activates the microcircuit in the chamber **3117**. As stated

above, this HF signal is rectified and filtered to provide power to the microcircuit. Additionally, a clock signal is removed from the HF signal radiating from the base antenna, and used to coordinate operations within the microcircuit to the operations within the housing and module **3068**. It should be appreciated that any other manner of exciting a non-powered microcircuit, or accessing identification data from a powered microcircuit, is contemplated by this disclosure. It is also appreciated that components suitable for use with other selected frequencies are also contemplated herein.

Once rectified, the microcircuit (rwd) is powered on and proceeds through its set-up routine which places the microcircuit in a condition wherein it can be programmed by the read/write controller. The read/write controller senses that the read/write device is not programmed and, as a result, selects an identification number for programming. As mentioned above, it is possible to program all of the microcircuits when the sleeves are made. In such an instance, the precoded information will be stored in a remote computer and associated with information regarding the contents of the vial or container. Thus, when the microcircuit is precoded,that identification code in incorporated into a database that identifies the contents of the cylinder for other container] with the identification code. As is customary in the industry, a variety of information fields could be used to accomplish a variety of informational purposes. Specifically, the identification code programmed into the microchip could be matched with a database that includes all patient information for the sample contained within the cylinder. For example, if the sample was blood, the database could include the patient's name as well as other aspects of the patient's file. In addition, in an automated process, the computer system where the database is maintained could be continually updated to monitor the current status of the sample or specimen contained in the cylinder associated with the particular sleeve and identification number.

Once programmed, the sleeve and vial can be removed from the carousel, or the carousel may simply be rotated so that a different sleeve may be inserted into a different port. In this manner, an entire carousel may be loaded and programmed within a short period of time. Despite requiring a short program period, the cylinder will never have to be re-programmed. In fact, many of the microcircuits are one-time-programmable (OTP) which removes any problem with erasing the identification code from the microcircuit. The vials with sleeve may be further processed in accord with methods described herein or other methods. The sleeve permits the vials to be readily identified and tracked.

The base antenna is substantially shaped like the sleeve antenna.

Specifically, The ratio between the diameter of the sleeve antenna and the base antenna should be approximately 1:1. In certain circumstances, the ratio can be as much as 3:1, where there is sufficient distance between the vial being identified and the neighboring vials. Matching the base antenna to the sleeve antenna is an important aspect of the inductive communication which passes between the two antennas. More particularly, in order for the communication between the two antennas to be optimized, the antennas must be properly matched and positioned. While it is not critical that the antennas be precisely matched, it is important to maintain the ratio of 3:1 or less for the difference in sizes between the two antenna.

A pair of leads **3071** pass from the base antenna to a filter board **3072** which is intended to adjust the frequency range of the base coil to match the frequency range of the sleeve coil. In the present embodiment where the base antenna and the sleeve antenna are similarly shaped, the filter board **3072** only includes a capacitive adjustment. In this particular embodiment, the capacitance needed to match the two antenna was on the order of 20 pF.

From the filter board **3072**, the electronic signal to and from the base antenna passes through wire **3080**. This wire handles all communications between the module **3068** and the read/write device. Inside the module is a circuit board which includes those components shown in FIG. **1** U.S. Pat. No. 5,345,231. More specifically, the circuit within the module includes an oscillator, modulator, demodulator, clock extractor, control unit, and interface.

In particular, the modulator and demodulator are matched to the modulator and demodulator housed on the microchip. The control chip essentially coordinates all activity between the computer system **3004** and the module [see, U.S. Pat. No. 5,345,231, which describes the operation of the circuitry within the module].

Wire **3070** passes from the control module **3068** to the computer system **3004**. This connection is a standard serial communication channel that is readily accepted into virtually all modern computer systems. In the present embodiment, the computer system is equipped with a program that is designed to receive the serial digital information from the module that represents the identification code of the microcircuit in the read/write device. In addition to decoding the identification number, the computer system can be provided with positioning information that identifies the location within the carousel of the particular sleeve and vial. Moreover, because the motor is electrically actuatable, the particular vial can be identified and repeatedly accessed without having to re-decode the identification code for each instance.

Although the positioning of the sleeves had been discussed in terms of being mounted on a containment device of one sort or another, it is to be appreciated that virtually anything could be identified with such a read/write device. More specifically, in the instance of the preferred embodiment, the carousel itself could be equipped with a read/write device that would enable the read/write controller to identify which carousel out of a number of carrousels was actually installed on the system **3000**. To this end, it is to be appreciated that the housing **3018** could be equipped with a number of base antenna that could simultaneously read a number of sleeve antennae and read/write device, as well as those read/write devices located within the carousel itself.

Referring now to FIG. **43**, a typical display **3200** is shown that is generated from software, such as software that one of skill in the art could design based on the disclosure herein. As shown, the screen has a number of graphical plots **3202**, **3204** that may be used to show any number of characteristics of the sample contained within the vial. In this display, the identification code **3206** is shown at the lower left as read from the read/write controller and decoded by the computer system **3004**. While this display shows various data fields, it is to be appreciated that virtually an unlimited number of data fields **3210** could be incorporated into a series of displays associated or linked with a single identification code. More particularly, the identification code could be linked to a database that indicates the contents of the cylinder as well as the location of the cylinder, as well as any number of other features that are also relevant to the contents of the cylinder. For example, the graphs shown here depict various levels of a substance which either varies over time, or following a series of tests. .

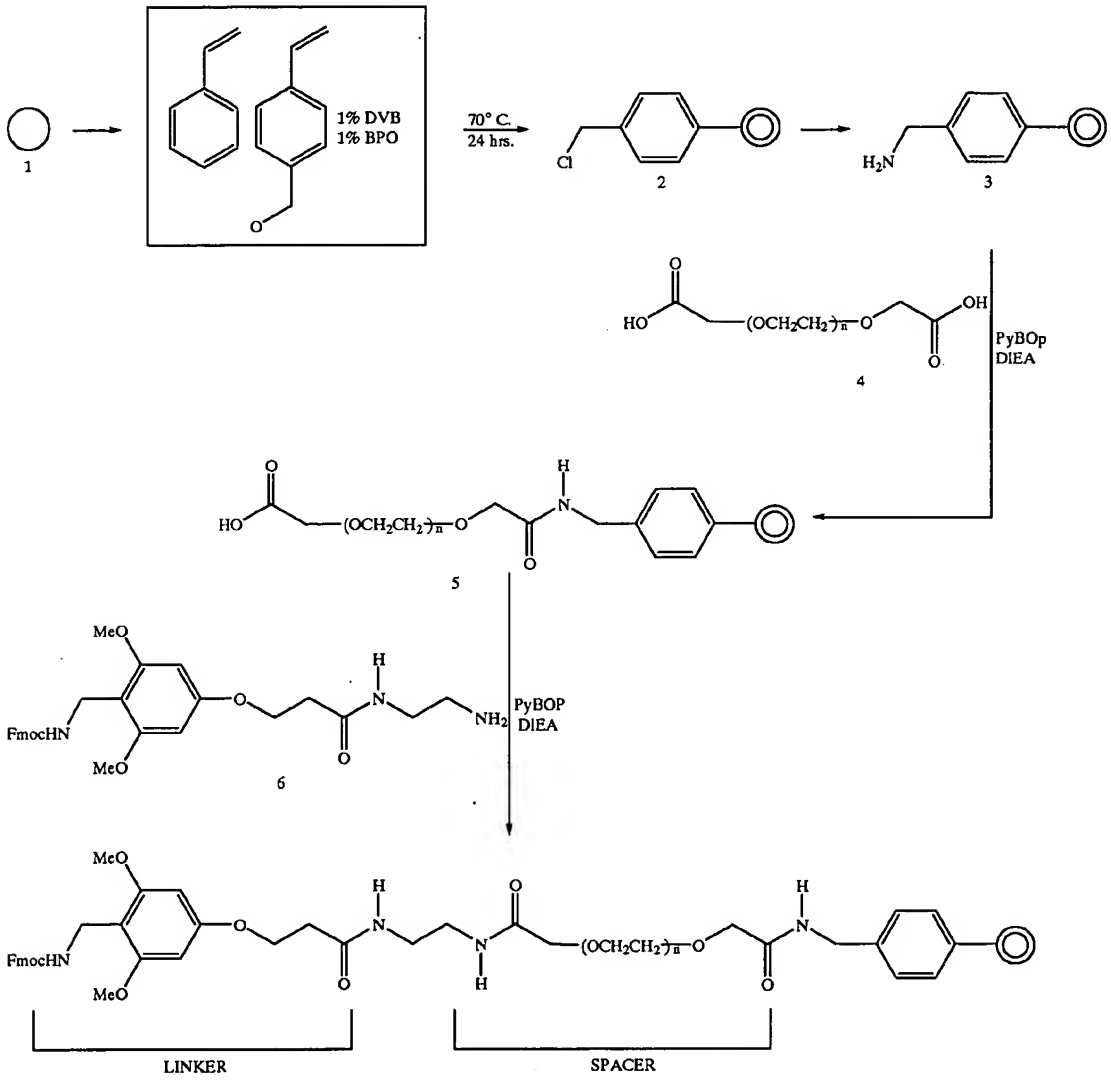
The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE 1

Formation of a Polystyrene Polymer on Glass and Derivatization of Polystyrene

A glass surface of any conformation [beads for exemplification purposes (1) that contain a selected memory device or that are to be engraved with a 2-D optical bar code or that include a 3-D memory] that coat the device or that can be used in proximity to the device or subsequently linked to the device is coated with a layer of polystyrene that is derivatized so that it contains a cleavable linker, such as an acid cleavable linker. To effect such coating a bead, for example, is coated with a layer of a solution of styrene, chloromethylated styrene, divinyl benzene, benzoyl peroxide [88/10/1/1/, molar ratio] and heated at 70° C. for 24 h. The result is a cross-linked chloromethylated polystyrene on glass (2). Treatment of (2) with ammonia [2 M in 1,4-dioxane, overnight] produces aminomethylated coated beads (3). The amino group on (3) is coupled with polyethylene glycol dicarboxymethyl ether (4) [n~20] under standard conditions [PyBop/DIEA] to yield carboxylic acid derivatized beads (5). Coupling of (5) with modified PAL [PAL is pyridylalanine]linker (6) under the same conditions produces a bead that is coated with polystyrene that has an acid cleavable linker (7).

The resulting coated beads with memories are then used as solid support for molecular syntheses or for linkage of any desired substrate.



EXAMPLE 2

Microvessels and Use Thereof

A. FIGS. 11–13

FIGS. 11–13 illustrate an embodiment of a microvessel 20 provided herein [see, also FIGS. 45-50 for an alternative embodiment]. The microvessel 20 is a generally elongated body with walls 22 of porous or semi-permeable nonreactive material which are sealed at both ends with one or more solid-material cap assemblies 42, 44. The microvessel

20 retains particulate matrix materials 40 and can, as depicted in the Figure, contain one or more recording devices 34 or alternatively is engraved or imprinted with a symbology, particularly, the 2-D optical bar code provided herein. In the embodiment illustrated in FIGS. 11–13, the recording device includes a shell 36 that is impervious to the processing steps or solutions with which the microvessel may come into contact, but which permits transmission of electromagnetic signals, including radiofrequency, magnetic or optical signals, to and from the memory engraved on or in the device.

The microvessel 20 is generally cylindrically shaped and has two solid-material cap assemblies 42, 44. The cap assemblies may be formed of any material that is non-reactive with the solutions with which the microvessel will come into contact. Such appropriate materials include, for example, plastic, TEFLON, polytetrafluoroethylene (hereinafter, PTFE) or polypropylene. Each cap assembly 42, 44 preferably includes a support base 26, 28, respectively, and an end cap 24, 30, respectively. Each support base 26, 28 is permanently attached to the walls 22 of the vessel by known means such as bonding with appropriate adhesives or heat treatment, either by heat-shrinking the wall material onto the lower portions of the support bases 26,28, or by fusing the wall material with the support base material.

Preferably, at least one of the caps 24,30 is removably attached to its cap base 26, for example by providing complementary threads on the support base and the end cap so that the end cap can be screwed into the support base, as illustrated in FIG. 12. Other possible means for attaching the end cap to the support base will be apparent to those in the art, and can include snap rings, spring tabs, and bayonet connectors, among others. The end cap 24, has one or more slots, bores, or recesses 32 formed in its outer surface to facilitate removal or replacement, with the user's fingers and/or by use of an appropriate tool. For the example illustrated, a spanner wrench having pegs spaced at the same separation as the recesses 32 can be used by inserting the pegs into the recesses. For a single slot, removal and replacement of the end cap could be achieved by using a screwdriver. Protruding tabs, rims, knurled edges or other means to enhance the ability to grasp the end cap can be used for manual assembly/disassembly of the microvessel. The cap assembly 42 at the opposite end of the microvessel can be permanently sealed using an adhesive or heat treatment to attach the support base 28 to the end cap 30, or the cap assembly 42 can be molded as a single piece, combining the support base 28 and the end cap 30.

Retained within the microvessel 20 are particle matrix materials 40 and, as depicted a memory device 34. In embodiments herein, the memory device will be a symbology engraved on the device, such as on the cap.

The illustrated microvessel, as illustrated in FIGS. 11–13, is of a size sufficient to contain at least one recording device and one matrix particle, such as a TENTAGEL™ bead. The device is typically 20 mm in length [i.e., the largest dimension] or smaller, with a diameter of approximately 5 mm or less, although other sizes are also contemplated. These sizes are sufficient to contain form about 1 mg up to about 1 g of matrix particle, and thus range from about 1 mm up 100 mm in the largest dimension, typically about 5 mm to about 50 mm, preferably 10 mm to 30 mm, and most preferably about 15 to 25 mm. The size, of course can be smaller than those specified or larger. The wall material of the microvessel is PTFE mesh or other chemically inert surrounding porous support [polypropylene AA, SPECTRUM, Houston, TX], or other suitable material, having a preferably about 50 μM to 100 μM, generally 50 to 70 μM hole size that is commercially available. The size of course is selected to be sufficiently small to retain the matrix particles. The cap apparatus is machined rod PTFE [commercially available from McMaster Carr, as Part #8546K11].

The matrix material is selected based upon the particular use of the microvessel; for example, a functionalized resin, such as TENTAGEL™ resin [e.g., TENTAGEL™ polymer beads carrying an acid-cleavable linker, from matrix material may also include fluophores or scintillants as described herein.

Alternative embodiments of the microvessel will be appreciated and include, for example, a pouch, including porous or semi-permeable material, which is permanently sealed to itself and contains matrix material and one or more memories.

About 20 mg of the derivatized TENTAGEL™ beads have been sealed in a small [of a size just sufficient to hold the beads] porous polypropylene microvessel (see, Examples, below].

In alternative embodiments, microvessels in which the tube (or other geometry) is solid (not porous), such as polypropylene, PTFE or other inert surface that has been radiation grafted, as described herein, may also be used. With these devices syntheses are performed on the surface and the solid tube is engraved with a symbol or includes an optical memory or other tag, which may be permanently or removably sealed inside. These devices, herein denoted the MICROTUBE microreacters (or microvessels), may be used in the methods herein interchangeably with the MICROKAN microreactor type of device. In addition, these may also be advantageously used in the optical embodiments.

The "microreactors" provided herein permit the advantageous productivity gains of the split-and-pool technique, without any of its limitations.

B. Tagging

By pooling and splitting matrix with memory microreactors [rather than individual solid phase resin beads] by a process known as "directed sorting", one discrete compound is synthesized in each matrix with memory reactor or microreactor. Each microreactor contains a memory, such as an optical memory, that is a unique label or tag used to identify it during the sorting processes that occur between chemical synthesis steps.

The memory tag provides a unique ID for each matrix with memory reactor and therefore each compound. This unique ID allows each microreactor to be identified during the combinatorial directed sorting process.

C. The "Directed Sorting™" Approach to Solid Phase Combinatorial Chemistry

The "directed sorting" approach to combinatorial chemistry is made possible by splitting and pooling matrix with memory microreactors rather than individual solid phase resin beads. During the first directed sorting step each microreactor is assigned to one specific compound. This assignment is maintained during all subsequent directed sorting and synthesis steps.

Tagging with a memory that is either engraved or imprinted during processing, subsequent to or pre-encoded [with decoding information stored remotely and associated with identifying information] of microreactors provides convenient and positive identification of compounds for archival and storage purposes. Such tagging permits the microreactors to be sorted between the individual steps in the synthesis.

Traditional split-and-pool methodology relies on a statistical distribution of resin beads between each step in the chemical synthesis. Typically, a large number of resin beads are used for each compound being synthesized to ensure an adequate statistical distribution of compounds. A consequence of this approach is that individual compounds are synthesized on multiple solid phase resin beads. These multiple copies of each compound are mixed together with multiple copies of all the other compounds. these mixtures need to be deconvoluted during screening. In contrast, the directed sorting approach ensures that:

1. Every compound is synthesized
2. Only one copy of each compound is synthesized
3. All compounds are present as discrete entities (no mixtures).

D. Software

Software, described herein, provides:

1. A repository for the chemical synthesis information—primarily the building blocks and reaction steps. Other information, such a pre-reaction procedures and reaction work-up procedures ban also be stored.
2. Explicit directions teaching how to sort the microreactors between each reaction step to ensure that all compounds, and no duplicates, are synthesized.
3. An interface from the chemical synthesis environment and format (individual compounds in microreactors) to the biological screening environment and format (cleaved compounds in 96-well microplates).

D. FIGS. **14–16**

FIGS. **14–16** illustrate an alternate embodiment of a microvessel **80** provided herein. Like the microvessel described in Example 3, this embodiment of the microvessel retains particulate matrix materials and can be imprinted with a symbology or will contain one or more recording devices (not illustrated). The microvessel **80** has a single-piece solid material frame **82**, including a top ring **84**, two support ribs **88, 89** disposed diametrically opposite each other and a bottom cap **86**. The solid material frame **82** may be constructed of any material which is non-reactive with the solutions with which the microvessel **80** will come into contact. Such appropriate materials include, for example, plastic, PTFE, TEFLON or polypropylene, and formation may be by molding or machining of the selected material, with the former being preferred for economy of manufacture.

The sidewall of the microvessel 98 is formed of porous or semi-permeable non-reactive material, such as PTFE mesh, preferably having a 70 μM pore size. The sidewall is preferably attached to the top ring **84** and bottom cap **86** of the solid material frame **82**. Such attachment may be by known means such as bonding with appropriate glues or other chemicals or heat, with heat being preferred.

In the embodiment of FIGS. **14–16**, the two support ribs **88, 89** are positioned opposite one another, however, any number of support ribs, i.e., one or more, may be provided. The microvessel sidewall **98** need not be fully attached to the support ribs **88, 89**, however, the molding process by which the microvessels are formed may result in attachment at all contact points between the frame and the sidewall.

In the preferred manufacturing process, the sidewall material, a flat sheet of mesh, is rolled into a cylinder and placed inside the mold. The frame material is injected into the mold around the mesh, causing the frame to fuse to the mesh at all contact points, and sealing the edges of the mesh to close the cylinder.

In the embodiment illustrated in FIGS. **14–15**, the microvessel **80** is configured with a removable end cap **90**. The end cap **90** is preferably constructed of the same material as the solid material frame **82**. A snap ring, or, as illustrated, projections **92, 94** extend downward from the inside surface of the end cap **90**. The projections **92, 94** have a flange which mates with a groove formed in the inner wall of top ring **84** when pressed into the top ring to releasable secure the end cap **90** to the microvessel **80**. As will be apparent, other means for releasably securing the end cap **90** to the top ring **84** can be used, including, but not limited to, those alternatives stated for the embodiment of FIGS. **11–13**. The dimensions vary as described for the microvessel of FIGS. **11–13** and elsewhere herein.

In other embodiments, these vessels fabricated in any desired or convenient geometry, such as conical shapes. They can be solid at one end, and only require a single cap or sealable end.

These microvessels are preferably fabricated as follows. The solid portions, such as the solid cap and body, are fabricated from a polypropylene resin, Moplen resin [e.g., V29G PP resin from Montell, Newark Del., a distributor for Himont, Italy]. The mesh portion is fabricated from a polypropylene, polyester, polyethylene or fluorophore-containing mesh [e.g., PROPYLTEX®, FLUORTEX®, and other such meshes, including cat. no. 9-70/22 available from TETKO® Inc, Briarcliff Manor, N.Y., which prepares woven screening media, polypropylene mesh, ETF mesh, PTFE mesh, polymers from W. L. Gore. The pores are any suitable size [typically about 50–100 μM, depending upon the size of the particulate matrix material] that permits contact with the synthetic components in the medium, but retains the particulate matrix particles.

EXAMPLE 3

Manual System

Illustrated in FIG. **17** is a program/read station for writing to and reading from the memory devices in the microvessel. The electronic components are commercially available from the same supplier of the memory devices, e.g., BMDS or ID TAG or the monolithic memory provided herein [Bracknell Berks RG12 3XQ, UK], so that the basic operations and frequency are compatible. The basic controller **170** and the transceiver **172** are disposed within a housing **174** which has a recessed area **176** positioned within the transmission range of coil **178**. The microvessel **180** may be placed anywhere within recessed area **176**, in any orientation, for programming and reading functions. Basic controller **170** is connected to the system controller **182**, illustrated here as a functional block, which provides the commands and encoded data for writing to the memory device in the microvessel and which receives and decodes data from the memory device during the read function. System controller **182** is typically a PC or lap top computer which has been programmed with control software **184** for the various write and read functions.

An example of the operation of the system of FIG. **17** is illustrated in FIG. **18**. When power is supplied to the system, transceiver **172** emits an interrogation signal **185** to test for the presence of a memory device, i.e., a responder, within its detection range. The interrogation signal **185** is essentially a read signal that is continuously transmitted until a response **186** is received. The user manually places a microvessel **180** within the recessed area **176** so that the interrogation signal **185** provides a response to the controllers indicating the presence on the microvessel. The system receives the interrogation signal and performs a decode operation **187** to determine the data on the memory device within the

microvessel, which data may include identification of the device and data concerning prior operations to which the microvessel has been exposed. Based upon the data obtained, the system makes a determination **188** of whether additional information is to be written. The system then performs a write operation **189** to record the immediately preceding operation. The write operation **189** involves modulating the transmitted signal as a series of "0's" and "1's", which are recorded on the memory chip, which typically has a 128 bit capacity. After completion of the programming step **189**, an error check **190** is performed wherein a second read signal is emitted to verify the data that was written for integrity and correct content. If the correct data is not verified, the system may attempt to perform the write operation **189** again. After verification of the correct data, if the microvessel is one that should proceed to another operation, the system controller **182** will display instructions **192** for direction of the microvessel to the next process step.

The read operation is the same as the beginning of the write operation, with the interrogation signal being continuously transmitted, or transmitted at regular intervals, until a response is received. The response signal from the memory device in the microvessel **180** is conducted to system controller **182** for decoding and output of the data that is stored on the memory device.

Software within the system controller **182** includes a data base mapping function which provides an index for identifying the process step associated with data written at one or more locations in the memory device. The system memory within the system controller **182** will retain the identification and process steps for each microvessel, and an output display of the information relating to each microvessel can indicate where the microvessel has been, and where it should go in subsequent steps, if any. After the data stored within the microvessel has been read, it is removed from the interrogation field and advanced to its next process step.

Software for aiding in the steps in combinatorial synthesis schemes has been developed. The software, which in light of the description herein can be written, facilitates the process of creating chemical libraries with the systems provided herein. The exemplified software, now available under the name ACCUTAG™ Synthesis Manager Software as a part of the AccuTag™-100 Combinatorial Chemistry System [e.g., an embodiment of the system provided herein]. These systems exemplified with the device of FIG. **17** [e.g., sold under the name ACCUTAG™], computer-based hardware, and the matrix with memories used therewith, such as the MICROKAN matrix with memory device and the MICROTUBE matrix with memory device [see, e.g., FIGS. **11–15** and **21**].

The software is organized into the following sections. These sections present the normal sequence of activities that go into building a library with the system provided herein.

1. Define Building Blocks. The user enters the names of the chemical building blocks to be used. For brevity of reference, a code letter is assigned to each building block.
2. Plan Steps.
   a. Number of Steps. The user specifies the number of steps. In a given step, a building block, such as a monomer, amino acid, nucleotide, will be chemically added to each compound that is being synthesized.
   b. Building Blocks To Use. The user specifies which of the defined building blocks will be used in each step. If, for example, there are 3 steps and the user specifies building blocks A, B, C in step 1, building blocks D, and number in step 2, and building blocks F, G, H, I in step 3, then the resulting library will contain 24 unique compounds because there are 3×2×4=24 combinations of building blocks.
   c. Procedural information. The user optionally enters "recipe" information such as reaction times, temperatures, molarities, and reagents to use for each building block's reactions as well as procedures common to all building blocks. At the appropriate times during the "Perform Synthesis" section of the program, this information is "played back" to the user. This is a convenience function for the user.
3. Perform Synthesis. Using a virtual library database of all the involved building blocks, reactions, process and compound tracking data, the software facilitates the step-by-step synthesis of the chemical library using memories with matrices, such as a MICROKAN OR MICROTUBE. For each step specified in Plan Steps (above) the following four tasks are performed.
   a. Pre-Procedure. Any preliminary procedures that the user entered are displayed. Typically these will involve chemical "deprotection" of the reaction site associated with this step.
   b. Sorting. The "directed sorting" process for the current step is administered by the software. The user is prompted to place a memory with matrix on the scanning station [see, e.g., FIG. **17**], which is connected to a computer. The memory in the matrix, i.e., the tags, identification [ID] is read. The software does a database look up, seeking this unique ID. On the first step, the tag's ID is not found in the data base, so the software assigns it to the first compound in the library, which has not yet been associated with a tag. The user is instructed to place the device into the reaction vessel for the appropriate building block. From this point on, when this tag is read, the user is instructed to put the device into the reaction that will add the building block planned for this step for this specific compound.
   c. Reactions. Through directed sorting, all the devices in the library are now in reaction vessels. These is one vessel for each building block in the current step. The user is now prompted to perform the synthetic chemistry that will add each vessel's building block to the compounds it contains. The software displays any procedure information pertaining to reaction conditions that the user entered in Plan Steps.
   d. Work Up. The user is prompted to perform the "work up" [follow-up] task. Any work-up procedures the user entered in Plan Steps are displayed. Typically these involve rinsing and drying the reactor devices.
4. Archive. Archive refers to the process of transferring the completely synthesized compound from matrices with memories to a storage medium, such as a 96 well microplate or vials of any shape or size.

This works as follows.

   a. User chooses either vials or microplates [or other container].

These containers or vials may include memories into which identifying information can be entered, such as by scanning the first memory and then entering the scanned information into the memory in the matrix [container] into which the compounds are transferred.

   b. User places device on memory with matrix reader, a scanning station [see, e.g., FIG. **17**].
   c. User selects a placement location: a well in a plate or a specific vial number.

d. User affirms placement location and the database is updated to document this. Chemically, the user typically cleaves the compound from the solid phase support and deposits only the synthesized compound in the storage media, while salvaging the reusable tag device for reuse on a another library.

e. The software automatically selects the next storage location. The user may override this, and make another selection.

While not required part of the process, additional functions, such as the following functions are provided.

1. Utility Functions.
   a. Decode Tags. Using this function, at any time, the user can place a tag on the Scanning Station. If the tag has been assigned to a compound in the library, then information about that compound is displayed.
   b. Find Compound. The user can specify a combination of building blocks. The software looks up this combination, and if it exists, it displays information about the compound and its tag.
   c. Status. Spreadsheets showing all devices, their building block assignments and process status (which steps have been sorted) is shown.
2. Printing. The user can print out report describing:
   a. Building Blocks
   b. Steps planned.
   c. List of All Compounds.
3. On Line Help. The user can get context-sensitive assistance and a hypertext version of the System's User's Guide.

EXAMPLE 4

Preparation of a Library and Encoding the Matrices With Memories

A typical matrix with memory, such as the MICROKAN matrix with memory reactor will provide the following yield:

Resin loading	0.5–1.0 μmol/mg resin
Using 30 mg of resin:	15–30 μmol compound
For a 500 MW compound:	7.5–15 mg of compound.

A pool of the matrices with memories prepared as in EXAMPLE 2 was split into two equal groups. Each group was then addressed and write-encoded with a unique radio frequency signal corresponding to the building block, in this instance an amino acid, to be added to that group.

The matrices with memories were then pooled, and common reactions and manipulations such as washing and drying, were performed. The pool was then re-split and each group was encoded with a second set of radio frequency signals corresponding to the next set of building blocks to be introduced, and the reactions were performed accordingly. This process was repeated until the synthesis was completed. The semiconductor devices also recorded temperature and can be modified to record other reaction conditions and parameters for each synthetic step for storage and future retrieval.

Ninety-six matrices with memories were used to construct a 24-member peptide library using a 3×2×2×2 "split and pool" strategy. The reactions, standard Fmoc peptide syntheses [see, e.g., Barany et al. (1987) *Int. J. Peptide Protein Res.* 30:705–739] were carried out separately with each group. All reactions were performed at ambient temperature; fmoc deprotection steps were run for 0.5 h; coupling steps were run for 1 h; and cleavage for 2 h. This number was selected to ensure the statistical formation of a 24-member library [see, Burgess et al. (1994) *J. Med. Chem.* 37:2985].

Each matrix with memory in the 96-member pool was decoded using a specifically designed radio frequency memory retrieving device [Bio Medic Data Systems Inc. DAS-5001 CONSOLE™ System, see, also U.S. Pat. No. 5,252,962 and U.S. Pat. No. 5,262,772] the identity of the peptide on each matrix with memory. The structural identity of each peptide was confirmed by mass spectrometry and ^1H NMR spectroscopy. The content of peptide in each crude sample was determined by HPLC to be higher than 90% prior to any purification and could be increased further by standard chromatographic techniques.

EXAMPLE 5

Synthesis of Oligonucleotide Libraries on OMDs

Oligonucleotide libraries are synthesized on OMDS [described above, see, e.g., FIGS. **22–30** and **33**]. Referring to FIG. **33**, polypropylene sheets [(10×10×1 mm) the Moplen resin e.g., V29G PP resin from Montell, Newark Del., a distributor for Himont, Italy) are radiation grafted with polystyrene to give the surface modified devices **1** [MACROCUBES™ or MACROBEADS™]. Each such device is imprinted with a unique symbology, such as the two-dimensional optical bar code using the methods described herein. The OMDs [also called laser optical synthesis chips] are then subjected [see, FIG. **33**] to a modified aminomethylation procedure [Mitchell et al. (1978) *J. Org. Chem.* 43:2845; Mitchell et al. (1976) *J. Org. Chem. Soc.* 98:7357; or other procedures to obtain other functional groups(Farrall et al. (1976) *J. Org. Chem.* 41:3877; Merrifield et al. (1985) *Angew. Chem. Int. Ed. Engl.* 24:7991)] to functionalize the polystyrene surface graft. Procedures are exemplified in the examples.

A laser optical memory device, shown in FIG. **33A**, was fabricated by combining two components: a 2-dimensional (2-D) 16 digit bar code for encoding and a separate polymeric support for synthesis. The 2-D bar codes were laser-etched by a CO_2 laser on 6×6 segments of a chemically inert alumina ceramic plate (Coors Ceramics, thickness=0.5 mm; the actual size of each 2-D bar code is 3×3 mm). The surrounding synthesis support is a stable polypropylene or fluoropolymer square (10×10×2 mm) radiolytically grafted (as describe herein) with low cross-linking polystyrene (Battaerd, G. W. Tregear, (1967) in Graft Copolymers, John Wiley & Sons, Interscience, New York) and designed with a square hole (6×6 mm) in the middle. The etched ceramic block is securely inserted within the hole to form the entire OMD. Very small size OMDS can be manufactured as the laser etching optical resolution of an entire 2-D bar code can extend well below 0.5 mm in total diameter. 2-D bar coding has the advantage over regular linear bar coding of more data compression in a much smaller surface area (FIG. **33B**).

A loading of a 5–8 μmol/device was typically obtained as measured by Fmoc analysis. At this point, the OMDS are ready for use in combinatorial or standard chemical synthesis.

A directed sorting strategy [instead of statistical pool and splitting] was used in the construction of combinatorial libraries with zero redundancy [i.e., the number of OMDs is equal to the number of the library members]. In an example of a 3×3 directed sorting synthesis [FIG **33C**), nine OMDs

are first scanned optically using a small camera [i.e., such as the QuickCam™]linked to the pattern recognition software [see, description above and FIG. 31] on a computer, and each device [with a unique 2-D bar-code], i.e., 1–9, for exemplification, is assigned to one of the nine members in the library [a Code-Structure Table] by the software that directs the synthesis, such as the Synthesis Manager software, described herein and in the EXAMPLES]. The OMDs are then split [sorted], using software for synthesis and for decoding the 2-D code pattern [see Appendix I and description herein] into three groups according to the first building block (A, B, or C) for each structure as pre-assigned in the Code-Structure Table. A reaction with building block A, B, or C is then performed on each specific group. The OMDs are then pooled, washed, subjected to common reactions, scanned and re-sorted into three new groups according to the second building block (A, B, or C). A second reaction with building block A, B, or C is then performed with each group of OMDs. The OMDs can then be pooled again, subjected to common manipulations, and sorted. The process is repeated until the synthesis is completed.

The structure of the compound synthesized on each OMD can then be de-coded simply by optically reading the 2-D image with synthesis software via the camera and the decoding software and correlating the bar code with the structures in the Code-Structure Table.

To demonstrate the utility of the laser optical synthesis OMDs in the large scale synthesis of oligonucleotides, a library of 27 oligonucleotides with a general structure of X_4-X_3-X_2-T was constructed using the above described Directed Sorting strategy (FIG. 33D). Since polystyrene does not completely swell in acetonitrile or water, which are the solvents for the coupling and cleavage steps in a standard oligonucleotide synthesis cycle[Gait et al. (1990) in *Oligonucleotide Synthesis, A Practical Approach*, Gait, Ed., IRL Press, Oxford], reaction conditions were modified to accommodate the polystyrene support. Among the range of solvents and co-solvents investigated, it was found that a mixture of acetonitrile and dichloromethane (2:3, v/v) for the coupling reaction gave the highest coupling efficiency, and water/1,4-dioxane (1:1, v/v) performed best as the solvents for the cleavage step. The standard conditions for the de-blocking step (3% trichloroacetic acid/dichloromethane) and the oxidation step (0.1 M I_2/THF) are directly applicable. All reactions [see FIG. 33D] were performed in appropriate size glass bottles with Teflon-lined screw caps, and all the washings are performed using acetonitrile and dichloromethane alternately.

Reaction conditions were as follows: de-blocking, 3% TCA/DCM, rt, 2 min, repeat 2 times, coupling, 0.1 M DMT-X_n, 0.3 M tetrazole, ACN/DCM (2:3 v/v), rt, 1 h; oxidation, 0.1 M I_2, THF/pyridine/H_2O (40:10:1, v/v/v), rt, 20 min; capping, 0.5 M Ac_2O, 0.5 M 1-methylimidazole, 0.45 M 2, 6-lutidine, THF, rt, 20 min.; cleavage, concentrated ammonia/1,4-dioxane (1:1 v/v), rt, 20 h; deprotection, concentrated ammonia, 55° C., 20 h,; de-salting (and de-blocking) on Poly Pak cartridges (according to manufacturer's instructions).

Twenty-seven amino-functionalized OMDs were first reacted with 0.1 M 5'-O-DMT-3'-succinic acid-2'-deoxythymidine/0.1 M PyBop/0.2 M DIEA/DMF (30 ml) at room temperature for 4 hours. The OMDs were washed (acetonitrile and dichloromethane alternately, 30 ml×4 for each solvent) and dried under vacuum at room temperature for 30 min (all subsequent reactions were followed by the same washing and drying procedures). The OMDs were then capped with Ac_2O/1-methylimidazole/2,6-lutidine/THF (0.5 M for each, 30 ml) at room temperature for 20 min., and de-blocked with 3% trichloracetic acid/dichloromethane (30 ml, rt, 2 min., repeated two times). UV measurement of the de-blocking solution indicated an average loading of 7.0 μmol per OMD. The OMDs were then scanned and each OMD was assigned to one of the 27 oligonucleotide sequences in the library (Code-Structure Table) using software, such as the Synthesis Manager software described herein, and a camera, such as the QUICKCAM camera. The OMDs were sorted into three groups according to the second residue assignment (A, G, or C) and coupled with one of the corresponding β-cyanoethyl phosphoramidites (0.1 M) in acetonitrile/dichloromethane (2:3, v/v, 10 ml, with 0.3 M tetrazole) at room temperature for 60 min. The OMDs were then pooled together, oxidized (0.1 MI_2/THF/pyridine/H_2O, 30 ml, rt, 20 min), capped, and de-blocked. Next, the OMDs were subjected to another cycle of sorting→coupling→oxidation→capping→de-blocking according to the third residue (X_3) in their assigned structures. After the third residue, the OMDs were scanned, sorted into three groups according to their last residues (X_4), coupled to the corresponding phosphoramidites, and oxidized. The capping and de-blocking steps were omitted in this cycle.

The washed OMDs were then scanned with the QUICKCAM™ and the sequence of the oligonucleotides on each OMD was de-coded. Each OMD was put into a 5 ml glass vial labeled with the corresponding oligonucleotide sequence. Concentrated ammonia and 1,4-dioxane (2 ml, 1:1, v/v) was added to each vial and the vials were sealed with Teflon-lined screw caps. The vials were shaken at room temperature for 20 hours to cleave the oligonucleotides from the support. The OMDs were then removed from the vials and rinsed with aqueous dioxane (0.5 ml×2) and the vials were evaporated to dryness under vacuum. Next, fresh concentrated ammoni a (2 ml each) was added to each vial. The vials were capped tightly and heated in an oil bath (55° C.) for 20 hours to removed the cyanoethyl, isobutyryl, and benzoyl groups. An aliquot (10 μl) of the crude oligonucleotide solution from each vial was saved for HPLC analysis and the rest of the solutions were evaporated under vacuum. The crude oligonucleotides were then de-blocked and de-salted with Poly Pak™ cartridges (Glenn Research) using standardized procedures[User Guide for Poly Pak Cartridges from Glenn Research]. The fully de-protected oligonucotides were lyophilized from 20% acetonitrile/water (white solids), weighed, and analyzed by MS, ^{1}H NMR:

[Selective ^{1}H NMR spectra data (500 MHz, D_2O). ACCT: s=8.47 (s, 1 H, CH-adenine), 8.37 (s, 1 H CH-adenine), 8.09 (d, J=7.8 Hz, 1 H, CH-cytosine), 8.06 (d, J=7.8 Hz, 1 H, CH-cytosine), 7.68 (s, 1 H, CH-thymine), 6.15–6.32 (3 multiplets, 6 H, CH-cytosine and O—C(N)H), 3.81–5.03 (6 multiplets, 16 H, O—CH_2 and O—CH), 2.28–2.92 (4 multiplets, 8 H, CH_2), 1.87 (s, 3 H, CH_3). ACAT: s=8.47 (s, 1 H, CH-adenine), 8.44 (s, 1 H CH-adenine), 8.35 (s, 1 H, CH-adenine), 8.34 (s, 1 H, CH-adenine), 8.05 (d, J=8.0 Hz, 1 H, CH-cytosine), 7.59 (s, 1 H, CH-thymine), 6.15–6.43 (3 multiplets, 5 H CH-cytosine and O—C(N)H), 3.78–5.1 (7 multiplets, 15 H, O—CH_2 and O—CH), 2.17–2.87 (5 multiplets, 8 H CH_2), 1.79 (s, 3 H, CH_3).]

The OMDs were also analyzed by sequence analysis. Oligonucleotide sequence analysis was performed using electrospray and EM mass spectrometry [ES MS-MS; see, e.g., Siuzdak (1966) in Mass Spectrometry for Biotechnology, Academic Press, San Diego; Metzger et al. (1994) *Anal. Biochem.* 219:261; Ni et al. (1996) *Anal. Chem.* 68:19891.

Each oligonucleotide gave the expected molecular ion in mass spectroscopy analysis as configured by the 2-D encoding. Sequence analysis of two oligonucleotides with the same molecular weight showed that they had the expected sequences. The crude products had good to excellent purity as analyzed by reverse phase HPLC. A quick, standard de-salting procedure using Ply Pak cartridges yielded 27 pure (>95% by HPLC) oligonucleotides with good overall isolated yields (while solid, 2–7 mg each). These data show that the combinatorial strategy of oligonucleotide synthesis using the laser optical synthesis (LOSC) technology should produce a large number and quantity of oligonucleotides with high purity.

EXAMPLE 6

Radiation Grafting of a Polymer on a Insert Surface for Preparation of Matrices With Memories

Matrices for use as supports for synthesis and for use in coupled [single platform] protocols have been prepared using radiation grafting. These supports include any inert surface, including PFTE [TEFLON™], which heretofore does not appear to have been used for radiation grafting. The methods exemplified below with reference to FIG. **34** have been designed for use with PFTE as well as other surfaces. A method of radiation-induced grafted copolymerization of styrene to Teflon (PTFF) has been developed.

A. Scheme 1

1. Preparation of polymer

Scheme 1 shows the preparation of polymer. Polystyrene is radiation grafted onto polypropylene or TEFLON® tubes, an RF tag, such as the BMDS tag, or IDTAG transponder, was inserted into the tube to produce what will be provided under the name MICROTUBE.

The polystyrene is then functionized with selected functional groups [i.e., such as "A" inf FIG. **34**A]. Scintillant is covalently linked onto the polystyrene though "A", and a bioactive molecules, such as, for example, biotin, can be synthesized on the surface using the remaining "A" functionalities.

2. Radiation

The teflon (PTFE) tube was radiated under a Co^{60} source at a dose rate of 0.1×10^5 r/h; the total dose is typically $2.6–2.9\times10^6$ r.

3. Polymers

Using radiation-induced grafting polymerization techniques, a variety of monomers such as styrene, acrylic acid, methylacrylic acid, 2-hydroxymethylacrylate, and other such monomers can be used to produce different polymeric surfaces with different functional groups on polypropylene (PP), polyethylene (PE) and fluoropolymers. Polyethylene oxide (PEG) may be grafted onto the surface to change the hydrophilicity and reduce the sterichinderance to antibodies or receptors. Functional groups such as amines, alcohols and phenols, carboxylic acids, halides, aldehydes, nitrites and other such groups, can be introduced.

It was found that dilution of monomers, such as styrene, with methanol enhanced the rate of grafting PP and PTFE tubes have demonstrated highest styrene grafting at styrene concentrations of about 25 to 50%.

4. Functionalization

The functionalization was performed using the readily available N-(hydroxymethyl) phthalimide, with trifluoromethanesulfonic acid as catalyst. The polystyrene grafted tubes is thoroughly washed before use to remove residual monomer, non-attached polystyrene and additives remaining from radiation grafting. The amidoalkylation proceeds smoothly in the 50% (v/v) trifluoroacetic acid—dichloromethane as solvent at room temperature for 24 hours. The predetermined loading can be obtained by changing the concentrations of reagent, catalyst and reaction time. The hydrazinolysis in refluxing ethanol gives the aminomethyl polystyrene grafted PTFE tube. The microtubes were prepared in different sizes (2–12 mm) with loading capacity range from 0.5–15 μmol per tube.

5. Fluorophores

The scintillants, which are chemical stable, were chosen to match the energy gap from radiation energy of radioisotopes. Scintillants such as 9-anthracenepropionoc acid, 1-pyrenebutanoic acid and their derivatives are matched to the energy transfer for different radioisotopes, in including ^{125}I, ^{3}H, ^{14}C and others. Care should be taken when selecting combinations of scintillants and radioisotopes to match so that energy transfer from isotope to scintillant is matched.

A portion of the functional groups were covalently linked to the mixture of primary fluor (**S1**, molecules that emit light following interaction with radiation) and secondary fluor (**S2**, wavelength shifter). Experiments were performed with mixture of **S1/S2** at the ratio ranging from 20:1 to 100:1 for **S1** and **S2** respectively, with optimum ratio of 40:1 for most of the experiments presented here. Conditions in which 20% to 80% of the functional groups were occupied with mixture of **S1/S2** were evaluated. The optimum number of the functional group linked to primary and secondary fluors for most of the experiments was 50%.

The remaining of the functional groups (20% to 80%) were used for chemical synthesis. Small molecules (e.g., biotin) were synthesized on the solid support as described in the scheme 2.

B. Scheme 2: Biotin synthesis

In order to reduce steric hinderance and improve the interaction of labeled biological target (e.g., ^{125}I-receptor), and depending on the size and nature of the small molecule, a different percentage of the functional groups was utilized for chemical synthesis, while the remaining functional group were blocked with Boc. The experiments indicate that optimum results are obtainable with 25% of the functional group dedicated for chemical synthesis.

1. Synthesis

Fmoc (Fmoc-Gly-OH) and Boc(Boc-Gly-OH) linked amino acids were used to control the loading of scintillants and remaining amines. The Fmoc groups were removed using 20 piperidine in DMF, and Boc groups were removed using 1:1 ratio of TFA and dimethylmethane. 50% amine groups were covalently linked to scintillants. The remaining 50% amine were used to synthesis biotin.

2. SPA Assay

The biological activity of small molecules synthesized on the surface of the tubular matrices with memories may be evaluated in a variety of scintillation proximity assay formats as described herein. For example, biotin and its derivative (2-imidazolidone-4-carboxylic acid) were synthesized on the tube and the binding characteristics of the synthesized molecules on the solid support to 125I-streptavidin in scintillation proximity assay were evaluated. The results demonstrated that biotin derivative (2-imidazolidone-4-carboxylic acid) that has much lower affinity for streptavidin exhibited a lower signal.

C. A a teflon tube [19 mm, long, OD:5 mm, ID:2 mm; see FIGS. **34**C and **34**D] is radiation grafted. It was found that dilution of styrene with methanol enhances the rate of grafting. Dilutions of from 5% to 70% were tested. The PTFE tube has the highest styrene grafting at a 50% dilution [in contrast, a polypropylene tube has the best performance

at 35% dilution. The teflon (PTFE) tube is radiated under Co^{60} source at a dose rate of 0.1×10^6 r/h; the total dose of $2.6–2.9\times10^6$ r.

Functionization is performed using N-(hydroxymethyl) phthalimide, with trifluoromethanesulfonic acid [TFMSA] as a catalyst. The polystyrene grafted PTFE tube is thoroughly washed before use to remove residual monomer, non-attached polystyrene and additives remaining from radiation grafting. The amidoalkylation proceeds smoothly in the 50% (v/v) trifluoroacetic acid-dichloromethane solvent at room temperature for 24 hours. The predetermined loading can be obtained by changing the concentrations of reagent, catalyst and reaction time. The hydrazinolysis in refluxing ethanol gives the aminomethyl polystyrene grafted PTFE tube.

FIG. **34** depicts the protocol for radiation grafting of polymers to the surface of TEFLON® [or other suitable surface]. FIG. **34C** depicts the preparation of a tubular devices in which the matrix is the radiation grafted PTFE and the memory is a transponder, such as the monolithic device provided herein, the BMDS transponder or IDTAG™ transponder [such as a MICROTUBE], described herein; and FIG. **34D** depicts the small chip [2 mm×2 mm×0.1 mm, see, FIGS. **51** and **51**] encased in a radiation grafted polyprolene or teflon ball [or bead, conical tube or other such geometry] with a screw cap or snap on lid [such as device provided herein that will be provided under the name MICROBALL or MICROBEAD or MICROTUBE]. These devices may have removable lids, such as a snap on lid, preferably a snap on lid, or a screw top, so that the memory device can be removed and reused, and can be added after radiation grafting.

Loading on the grafted tubes and balls is adjustable can was typically about 0.5–15 μmol per tube. The amount can be varied by altering the size of the tube or balls. A variety of selected functional groups are available. Any known to those of skill in the art may be used, including any described herein.

PFTE devices are particularly suitable for high temperature reactions [loading was less than or about 3 μmol per device].

EXAMPLE 7

An exemplary glucose sensor with memory is set forth in FIGS. **54–59** and described in this example. This sensor unit which includes a memory may be programmed to collect data on glucose levels at a user defined schedule and to transmit the collected data to an external reader on demand, providing instant access to the most recent glucose history for the patient. In addition, the patient's prescribed treatments (e.g., insulin injections) can be stored in the sensor along with information about actual insulin injections and may be retrieved remotely. This information is available to the attending physician even without access to the patient's usual records. This can be very useful, for example, in cases for very young or old patients or for patients who are impaired due to hypoglycemia.

The most common method of measuring glucose is to use the enzyme glucose oxidase (GOx) to catalyze the reaction of glucose and oxygen to form hydrogen peroxide and gluconic acid:

$$\text{glucose}+O_2 \xrightarrow{GOx} H_2O_2+\text{gluconic acid}$$

Given a sufficient oxygen supply, the concentration of glucose is proportional to either the consumption of oxygen or the production of hydrogen peroxide. Hydrogen peroxide, however, tends to inactivate the glucose oxidase thereby shortening the sensor lifetime. To remedy this, the enzyme catalase can be used to break down the hydrogen peroxide

$$H_2O_2 \xrightarrow{catalase} \tfrac{1}{2}O_2+H_2O$$

so that the overall reaction is

$$\text{glucose}+\tfrac{1}{2}O_2 \xrightarrow{enzymes} \text{gluconic acid}$$

Because of the very strong buffering capacity of physiological solutions, any pH change from this reaction is typically very small.

Incorporation of this process for use as a sensor for detection of glucose concentration requires two oxygen sensors. The first oxygen sensor measures the ambient oxygen concentration surrounding the sensor. The second oxygen sensor is covered with a membrane or gel layer that contains the enzymes glucose oxidase and catalase. Oxygen and glucose diffuse into this layer and react according to the above reactions. Any excess oxygen remaining after the reaction is detected by the second oxygen sensor. The glucose concentration is proportional to the decrease in oxygen as measured by the difference between the two sensors.

A drawback of this method is that the oxygen concentration in living tissue is roughly two orders of magnitude less than that for glucose (0.01–0.15 mM for oxygen to 1–20 mM for glucose). Thus, once the available oxygen has been consumed, the above reaction is no longer sensitive to changes in glucose. This has been termed the "oxygen deficit" problem.

To address the oxygen deficit, sensing methods have been devised that do not rely on the use of glucose oxidase. Such methods include: optical methods (IR absorbency, competitive dextran binding) or the direct electrocatalytic consumption of glucose. Attempts have been made to "replace" the function of oxygen by using either electron carrier mediators, such as ferrocenes, or by directly coupling the glucose oxidase to the electrode in "wired" electrodes.

There are numerous techniques for dealing with the oxygen deficit by limiting the diffusion of glucose relative to that of oxygen. For example, in one method a special membrane is placed over the enzyme membrane that limits glucose diffusion. A second technique uses a geometrical arrangement that allows oxygen to diffuse everywhere but only allows glucose to diffuse through a small opening.

The process of sensing oxygen in a solution is commenced by a chemical reaction initiated electrically by one or more electrodes. When the potential of a noble metal electrode, e.g., Pt and Au, is made suitably negative, e.g., −500 mV with respect to a solution, oxygen at the surface of the electrode is reduced by the following reaction:

$$O_2+4H^++4e^-4e^- \rightarrow 2H_2O.$$

The removal of oxygen leads to the diffusion of oxygen to the surface of the electrode. The electrode current is proportional to the flux of oxygen with

$$\text{Flux}=D(\Delta[\text{oxy}]/\Delta L)$$

where Δ[oxy] is the difference in oxygen concentration between the electrode and the surrounding media or bulk concentration. Because the concentration of oxygen at the electrode is zero, [oxy] is equal to the outside bulk oxygen concentration. ΔL is the distance from the electrode surface at which oxygen is depleted to where the oxygen concen-

tration is equal to the bulk oxygen concentration (the oxygen gradient boundary layer region) and D is the constant of proportionality or diffusion constant for oxygen in an aqueous media. If the boundary layer, ΔL, is held constant then the electrode current is proportional to the oxygen concentration alone.

To maintain a constant potential between the solution and the oxygen sensing electrode, a three electrode potentiostat is used which contains a working electrode (where the oxygen is reduced), a reference electrode (which maintains a constant potential with respect to the solution), and a counter electrode. Because current flow through the reference electrode compromises its function, a counter electrode is provided as a current return path for the current flowing through the working electrode.

Glucose Sensor #1

FIG. **54** provides a diagram of a simplified circuit, with working electrode **542**, counter electrode **544**, reference electrode **546**, and amplifiers **548** and **549**. Working electrode **542** is typically platinum because it provides the most stable long term operation. Gold is also a common choice. Reference electrode **546** is usually made of silver with a coating of silver chloride to form a conventional silver/silver chloride electrode. For convenience, the counter electrode **544** is usually made of the same material as the working electrode, e.g., platinum or gold.

The oxygen reduction and the glucose oxidase reaction are temperature dependent. Therefore, for accurate measurements, it is necessary to monitor the temperature and compensate for any changes in temperature. To measure temperature, a thermistor chip is placed in close proximity to the sensing electrodes. The thermistor circuit **550** is a simple constant current circuit with changes in the measured voltage proportional to the changes in resistance of resistor **552** caused by temperature, as illustrated in FIG. **55**.

FIG. **56** is a block diagram of the major components of an exemplary implantable glucose sensing system, which may be broken down broadly into the electrical components **560** and the sensor electrodes **576**. The various components are discussed in more detail below. FIG. **57** is a cross-section of an exemplary implantable device with electronic components on the top half of the printed circuit board (PCB) **572** and the batteries **574** on the bottom with the sensor electrode assembly protruding **576** from one end of the PCB **572**.

The PCB **572** may be on the order of about 50 mm×50 mm (2"×2"), but is preferably smaller.

The basic electronic circuit is provided in FIG. **54**. A perspective diagrammatic view of the electrode section **576** of the electrode section of the sensor assembly **576** is provided in FIG. **58**. The electrodes **542,544,546** are formed by photolithographic patterning of thin-film metals deposited on both sides of a silicon or ceramic substrate **588** using conventional semiconductor processing techniques such as evaporation or sputtering. The substrate **588** is thin 10.2 mm (0.01") or less], also in accordance with standard semiconductor processing techniques. Where silicon is used, the substrate **588** is preferably coated with a silicon nitride (Si_3N_4) layer for passivation. The approximate dimensions of the base metal electrodes, on both sides of the substrate **588**, in the exemplary embodiment are as follows: counter electrode **544**: thickness of 100 Å Ti/3000 Å Pt, 0.13 mm (0.005") wide by 1.78 mm (0.07") long; working electrode **542**: thickness of 100 Å Ti/3000 Å Pt, 0.08 mm (0.003") wide by 1.78 mm (0.7") long; reference electrode **546**: thickness of 100 Å Ti/5000 Å Ag/2000 Å AgCl, 0.13 mm (0.005") wide by 1.78 mm (0.07") long, space apart from each other by 1.0 mm (0.004"). The substrate **588** dimensions are approximately 2 mm long (0.078"), 0.8 mm (0.03") wide, and 0.2 mm (0.008") thick, including the passivation layers. In each electrode, 100 Å titanium layer is provided for adhesion. In the reference electrode **586**, the top 3000 Å of silver is converted to silver chloride by exposing the silver layer to a dilute ferric chloride solution.

The electrodes **542,544,546** are connected to the main circuitry by means of tape automated bonding (TAB) a polyimide flexible circuit **5810** to the sensor and the main PCB **5812**. (Note that the actual conductors are not visible in the figure since they are sandwiched between the substrate **582** and the flexible circuit **5810**. A low viscosity polymer encapsulant **5812** is used to encapsulate the connection between the flexible circuit **5810** and the metal electrodes **542,544,546**. A hydrophilic electrolyte membrane **5814** is deposited onto the metal electrode surfaces on both sides of the substrate **588**. Electrolyte membrane **5814** contains a phosphate buffer, pH 7.4 solution to provide stable contact between the counter, working, and reference electrodes **542,544,546**. Electrolyte membrane **5814** is typically polyhydroxyethylmethacrylate or polyacrylamide. The membrane **5814** is soaked in the phosphate buffer.

The entire assembly, excluding the tape connections to the PCB **572**, is encased in a sheath **5816** of silicone rubber. A cavity **5818** over the metal electrodes **542,544,546** is formed on the top side of the substrate **588**. Finally, the cavity **5818** is filled with a glucose oxidase/catalase membrane **5820**. Membrane **5820** is composed of an aqueous solution of albumin, glucose oxidase and catalase which is crosslinked in a 25% glutaraldehyde solution.

Given the close proximity of electrode assembly **542,544, 546** and PCB **572**, it is sufficient to measure the temperature on the PCB. This is accomplished by use of a standard surface mount NTC thermistor **552** such as a NHQ 103R10 from Thermometrics, which is run using the constant current circuit illustrated in FIG. **55**.

Referring back to FIG. **56**, a microprocessor **562** which is used to control the sensor must be selected for low power operation and include multi-channel analog-to-digital capability. The PIC 16C71 is a low power microprocessor running at 32 kHz with four 8-bit AID channels. Separate, interconnected integrated circuits can be used for the microprocessor and the A/D converter, however these will likely occupy a larger area of the PCB, limiting the miniaturization of the sensor.

When activated, the microprocessor **562** first checks for requests for data from the external transmitter (not shown) that are being received via antenna **564** and RF modem **566**. If no request is present, microprocessor **562** reads the data from the three input channels, i.e., the two oxygen sensors and the thermistor **542,544,546**, computes a glucose concentration and stores these values in memory **568**. The memory **568** operates in a scrolling format such that the oldest written data is lost. The software flow is provided in FIG. **59**.

To conserve power, microprocessor **562** is turned off when inactive and no data storage or data reading is being conducted. A special, very low power (0.1 A) capacitive charging circuit **565** is used to time sampling. It is understood that one of skill in the art could readily, in light of the disclosure herein, substitute a software-based timing controller for the capacitive charging circuit. When the circuit **565** is sufficiently charged, microprocessor **562** is activated. Circuit **565** is preferably tuned to a frequency of between 10–20 seconds per sample.

As illustrated in FIG. **59**, in step **592**, the sensor's power is turned on in response to a signal initiated by the sampling

timer **565**. If a signal is received from the external reader (step **594**), microprocessor **562** will first provide a signal to the RF modem **566** and antenna **564** to transmit the sensor's identification and basic status, such as serial number, offsets and calibration data [e.g., temperature corrections (step **595**)]. Then, in step **596**, the contents of memory **568** are transmitted, after which the microprocessor **562** again checks to see if additional data is requested by the external reader (returning to step **592**). If no external data request is made, the current readings from the electrodes are collected and converted by microprocessor **562** into digital data in step **598**. (The signal generated by the electrodes is analog.) In step **5910**, the digital data is stored in memory **568**. If all memory locations are full, the oldest data will be overwritten. After writing, the sensor's power is turned off until the next timing cycle begins.

Collected data will be stored in memory unit **568**, which can be either a 2 KB EEPROM unit, such as a Microchip 24C16, or several 256 byte RAM units. The EEPROM uses less space but more power than the RAM chips. The EEPROM retains its memory even without power and, thus, does not create a power drain when microprocessor **562** is powered down. (Memory retention following complete loss of power, i.e., a dead battery, is of little value since an implant without power is no longer useful.) As previously described, a wireless transmitter is used to transfer data collected by the sensor to a remote reader. In FIG. **56**, transmitter **5610** is illustrated as an antenna coil **564** and RF modem **566** combination, but may be any antenna configuration designed to transmit the contents of memory along with implant specific data such as an identification number, offsets and the like, in response to an inquiry signal from an external transceiver (not shown) or at regular time intervals. Transmitter **5610** has a range of at least 152 mm (6") between the implant and external transceiver.

Power for all sensor functions is supplied by one or more encapsulated batteries **574**, shown in FIG. **57**, which may, for example, be the lithium thin package 3CXM 3V battery from Gould Electronics. Preferably, a magnetic switch **5616**, shown in FIG. **56**, is included to allow the battery **574** to be disconnected from the rest of the circuit during inactive or storage periods, as determined by the sleep timer **565**. The battery **574** and the magnetic switch **5616** may be mounted on the underside of PCB **572**. (Only the battery **574** is illustrated in FIG. **57**.)

The electronics portion of the implant is encapsulated within an inert, non-porous material **578** to protect it from the external environment. In the exemplary embodiment, the implant is placed in a mold fixture with the outward end of the sensor assembly with the sensor electrodes **576** protruding from the mold. The mold is filled with a low viscosity epoxy such as Stycast **1266** from Emmerson and Cummings. A vacuum is pulled on the mold to remove any entrapped air bubbles.

In order to minimize adverse tissue response to the implant, the implant is coated with a biocompatible matrix material **5722** that is known to be generally inert and well tolerated by the tissues. Examples of these materials are silicone rubber or a collagen/albumin membrane. As described elsewhere herein, an angiogenic factor, such as a growth factor or interleukin, may also be incorporated into the matrix material.

Fabrication of the exemplary sensors includes a first stage of mounting the various integrated circuits and other discrete components, shown collectively in FIG. **57** as surface mount components **560**, which include all of the electronic components of **560**, except for the battery **574**. The battery **574** is attached to the underside of the PCB **572**, but is not yet connected. Correct operation of the surface mount components **5710** is tested using an external battery supply (not shown) to prevent unnecessary battery drain.

The second stage of fabricating the sensors is formation of the sensor assembly [electrode] **542,544,546**, as previously described, up to the assembly step prior to addition of the glucose oxidase/catalase membrane. The electrodes **576** are then attached to the PCB **572** using polyimide tape **5712** in accordance with conventional TAB techniques. This attachment means provides a good electrical connection while providing a level of flexibility between the electrodes **542, 544,548** and the rest of the implant. Again using an external power source, a "burn in" process is run on the sensors to stabilize them and test for proper performance.

After correct operation of all components is verified, the battery **574** is connected and the assembly is tested again to check for proper system performance. After testing, the assembly including the PCB **572**, surface mount components **560** and battery **574** is encapsulated as described as above, with the electrodes **542,544,546** left outside of the mold.

The glucose oxidase/catalase membrane **5820** is added into the cavity **5818** in the silicone rubber sheath **5816** over the top electrodes using a glutaraldehyde crosslinking solution. After the membrane **5820** is crosslinked, the complete implant is rinsed in sterile solution. The matrix material **5822** is then added by spray or dip coating the entire outer surface of the implant except for the axial end of cavity **5818** where the glucose oxidase membrane **5820** contacts the solution. After completion, the unit is sterilized using gamma or e-beam radiation or ETO. From this point, the implant must be handled under sterile conditions. A pre-implant calibration is performed and this information is downloaded into the implant memory, making the implant ready for implantation.

EXAMPLE 8

Intracranial Pressure Monitor

Monitoring of intracranial pressure is indicated when intracranial swelling presents the risk of injury or death in acute situations (head trauma) and chronic conditions (hydrocephalus or brain tumor). The primary methods of intracranial pressure monitoring, including intraventricular catheter, subarachnoid screw or bolt, and epidural sensor, are invasive. The monitor is inserted through the skull, and requires an external, wired connection to a storage and display device. While most acute situations require only short-term monitoring where hard-wired connections are generally tolerated, chronic conditions, which would be better controlled with regular, long-term monitoring, present more of a problem.

The pressure sensor provided herein solves this problem. In a manner similar to the glucose monitoring embodiment of the recording device, a pressure sensor that produces an electrical signal is connected to an electrical circuit similar to that illustrated in FIG. **56**, i.e., electrical assembly **560**, with the distinction being that the electrodes are replaced by a pressure microtransducer **602**, as shown in FIG. **60**. Such microtransducers for Iintracranial pressure monitoring are known and are commercially available from a number of different manufacturers: Camino Laboratories of San Diego, Calif., Codman & Shurtlef Inc. of Randolph, Mass., and InnerSpace Medical of Irvine, Calif.

This microtransducer is typically an optical pressure sensor that includes a small displacement diaphragm **604** in

front of two optical fibers. Light is supplied through one of the fibers and is reflected by the displacement diaphragm into the second fiber where it is directed to an intensity-sensitive optical detector near the end of the second fiber. The intensity of the reflected light provides an indication of the amount of displacement of the diaphragm. Since the commercially-available optical transducers are still typically disposed within the end of a catheter that is connected to some external device, in order to make the sensor independent of external physical connections, an LED flight emitting diode) light source **606**, and a multi-wavelength optical detector **608**, are included on the PCB **6010** with the other drive electronics, logic and communication (telemetry) components, so that the LED **606** and the optical detector **608** are in optical communication with the optical fibers **6012** and **6014**, and so that no external connection is required.

Other non-optical pressure transducers are available and can be substituted for the above-described optically-based pressure transducer. For example, the NPC-109 disposable medical pressure sensor from Lucas Nova-Sensor would be a suitable alternative. In such embodiment, the sensing element changes ersistance with pressure and one member of a wheatstone bridge. The measurement is made by applying a fixed voltage e.g., 4 volts, and measuring the output voltage, which will be on the order of about 30 microvolts per millimeter mercury.

The microtransducer is mounted inside an axial bore through a small bone screw **614**, as illustrated in FIG. **61**, so that the transducer is exposed through a small channel **616** in the tip of the screw **614**. The back end **618** (not shown) of the screw holds the necessary drive electronics, logic and communication (telemetry, i.e. the RF or other remote communication circuitry) components, including, the LED light source **606** and optical detector **608**.

The intracranial pressure sensor, contained within screw **614**, is inserted through a burr hole that is drilled through the patient's skull so that the channel **616** at the tip is exposed to the desired location within the skull, depending on the desired monitoring site (ventricular, subarachnoid, or epidural).

As with the glucose monitor, the frequency of data collection can be user-defined by pre-programming the microprocessor, with the data being stored in the sensor's memory until the data is requested by the physician using an appropriate remote transceiver.

Other types of pressure monitors may be used in place of the microtransducers, such as strain sensors. Optical strain sensors use several methods including optical fiber Bragg gratings (FBG), stimulated Brillouin scattering, and polarimetry in birefringent materials. The FBG is the most readily available type of strain sensor, and is formed by writing a Bragg-type refractive index grating **624** into a single-mode germanium-doped silica fiber **622**, as shown in FIG. **62**. (The Bragg grating is shown diagrammatically as a sawtooth pattern.) Strain is detected by monitoring the reflected wavelength from the grating **624** as it is subjected to elongation or compression. As above, for a remotely accessible sensor, an LED (light emitting diode) light source **626**, and a multi-wavelength optical detector **628**, are included on the PCB **6210** with the other drive electronics, logic and communication (telemetry) components **6212**, so that the LED **626** and the optical detector **628** are in optical communication with the grating **624**. Since FBGs are temperature sensitive and compensation may be required, the same temperature measurement circuitry as provided for the glucose sensor embodiment is included.

Optical pressure sensors based upon microbending are also known and can be constructed with an optical fiber sandwiched between two opposing serrated plates that bend the fiber when pressure is increased, causing an intensity loss in light transmitted through the fiber. Again, such a sensor requires a light source and a light detector in order to be able to function without external physical connection. Fabry-Perot interferometric devices are also known for their ability to measure pressure changes. These can be constructed by bonding a silicon membrane onto the end of an optical fiber, and are available from Photonetics, Inc. This particular type of sensor requires two light sources with different wavelengths which, although making the device quite accurate, may impose a limitation on the ability to miniaturize the device.

EXAMPLE 9

Blood Urea Sensor

Chronic dialysis treatments for patients with kidney disease must be monitored to determine their efficacy in removal of toxins, including urea, from the patient's blood. Current monitoring methods require the patient's blood to be drawn for laboratory processing to measure urea concentration, with the data being processed either manually or by computer before any adjustments can be made in the treatment.

Although there are about thirteen dialysis treatments each month, blood chemistries are typically measured only once a month, resulting in long delays in modifying therapy prescriptions for patents. Also, each treatment session cannot reveal changes in the patient's condition that occur throughout the month. A device for real time monitoring of blood urea level during dialysis would permit individualized prescription and nutritional therapy for patients on hemodialysis and reduces the need for additional blood samples and laboratory analyses.

To provide such a device, the logic, power and communications (i.e., telemetry) components, as previously described, are combined with a urea sensor which is connected in-line with the dialysate effluent coming from the hemodialysis cartridge. The decrease of urea throughout the treatment can be correlated to the amount of urea removed.

As shown in FIG. **63**, the urea sensor includes a pair of pH sensors **632,634** mounted in a "T" connector **636** that is placed between the hemodialysis cartridge dialysate output port **638** and the normal tubing **6310**. One of the pH sensors **632** is covered by a membrane layer **6312** containing the enzyme urease. The urease hydrolyzes the urea to form carbon dioxide and ammonia, in accordance with the following reaction:

$$(NH_2)_2CO+H_2O \rightarrow 2NH_3+CO_2$$

The resulting change in pH within the membrane **6312** is indicated by the change in potential (in millivolts) as a function of the concentration of urea. The second pH sensor **634** serves as a baseline to remove the effect of changing sample pH. The sensors **632,634** may be conventional electrodes or pH sensitive ISFETs (ion selective field effect transistors), such as those described in Chapter 7 ("Semiconductor Field Effect Devices by F. Winquist and B. Danielsson) of *Biosensors—A Practical Approach*, edited by A. E. G. Cass, IRL Press, 1990.

The power, logic and communication (telemetry) components **632** of the sensor are much the same as those described above for the glucose sensor of Example 7, including the

memory device and microprocessor. Included within the memory device can be the patient's identification, prescribed treatment, and urea monitoring results. The sensor will store the results of each treatment and this treatment history would be available on demand or as a monthly report. The memory device can be sealed into a wall of the "T"-connector **636**, or can be a plug that is inserted through a dedicated opening in a wall of the connector, in either case, positioned so that the sensor is in direct contact with the fluid.

EXAMPLE 10

Free Calcium Sensor #1

The concentration of free calcium (Ca^{++}) in the blood, i.e., calcium that is not bound to proteins, can be used to detect and monitor a number of bone diseases or calcium regulation disorders (thyroid or kidney diseases). The test for free calcium typically involves drawing blood from a vein, then running laboratory tests to measure the amount of free calcium. The accuracy of such tests is affected by the patient's consumption of food or ingestion of or from administration of medications containing calcium within several hours prior to drawing the blood. The tests, thus, can provide incorrect results if the patient has not been careful about what was eaten, or is unaware that a particular food could effect calcium blood level. Long term periodic monitoring will be helpful not only in avoiding the need to repeat tests when the results were not as expected, but also to determine the rate of change in calcium blood level over extended periods of time to determine whether the patient's condition was worsening or improving.

Fluorescence-based techniques can be used to measure calcium concentrations, and such techniques have reportedly made measurement of intracellular calcium "routine" [see, e.g., Czarnik, (1995) "Desperately Seeking Sensors" *Chemistry & Biology* 2:423–428.] Calcium-sensitive fluorescent agents, such as fura-2 and indo-1 are not consumed, sensing, and, thus, can be reused if the sensor material has not been irreversibly commingled with the test sample. These, thus, can be used as in sensors for measuring calcium.

A memory device, such as any of those described here, can be constructed can be implanted for in vivo monitoring of calcium concentration in the blood, or can be sealed into a vial or other container in which blood to be analyzed is received for in vitro monitoring. An optical detector, similar to that described for Example 8, will be connected to the power, logic and communication electronics. Unlike the sensor of Example 8, a light source will be selected to emit light not at the same wavelength as that detected by the detector, but rather at the wavelength necessary for excitation of the fluorosensor [fluorophore]. The detector is then selected to detect light at the wavelengths at which the fluorosensor fluoresces.

The entire sensor structure can be encased in a protective shell formed of an optically transparent plastic or polymer, so that light can exit and enter the shell. The exterior of the shell is coated with a matrix which contains the fluorosensor. The matrix may be a latex (see, e.g., Slomkowski, et al. (1995) "Two-dimensional Latex Assemblies and Their Potential Application in Diagnostics, *TRIP* 3:297–304) or collagen or other suitable, preferably biocompatible support material. It may be desirable to optimize the amount of light directed toward the detector by positioning a ring or other structure with an interior reflecting surface around the outside of the shell. The reflector must be sufficiently open in structure to permit the analyte, Ca^{2+} to flow through it to come in contact with the fluorosensor, which event will be recorded by the photodetector and memory.

EXAMPLE 11

Sol-gel Encapsulated Biomolecules and Memories and Sol-gel Sensors

Sol-gel biosensors are known [see, e.g., Dave, et al. (1995) "Sol-Gel Encapsulation Methods for Biosensors", *Analytical Chemistry* 67:1120] for a description of preparation of a suitable sol gels. Briefly, the sol-gel can be a porous silicate glass formed by hydrolysis of an alkoxide precursor followed by condensation to yield a polymeric oxo-bridged SiO_2 network. The corresponding alcohol molecules are liberated during the process. The initial hydrolysis and polycondensation reactions in a localized region lead to formation of colloidal particles. The suspension containing these particles is the "sol". As the interconnection between the particles increases, the viscosity of the sol starts to increase and leads to the formation of a solid gel. The nature of the polymeric gel that results can be regulated to a certain extent by controlling the rates of the individual steps. As long as solvent remains in the structure, the gel continues to change, with the pores becoming smaller and the gel strength increasing as the structure ages. Most of the water and methanol liberated as a result of hydrolysis is retained in the gel. As the water evaporates, the gel material shrinks to approximately one-eighth the original gel volume. Once the gel shows no further loss of pore liquid under ambient conditions, it can be considered stable.

For biomolecular encapsulation in sol-gel, a buffer is added to increase the pH to biologically compatible values. The protein to be encapsulated is added to the sol after partial hydrolysis of the precursor. As the structure ages, the pores close around the protein molecules, trapping them inside. This trapping also ensures that the protein molecules are distributed uniformly throughout the structure and are isolated from each other. Reactions of the encapsulated proteins with the analyte are limited to single molecules, optimizing efficiency and simplifying calculation of maximum sensitivity for a given amount of protein.

Free Calcium Sensor #2

Using a similar memory structure to that described in Example 10, a calcium sensor can be fabricated using calcium-dependent photoproteins, such as the Aequorin photoprotein.

The photoprotein, aequorin, isolated from the jellyfish, Aequorea, emits light upon the addition of Ca^{2+} or other metal ion. The bioluminescence photoprotein aequorin is isolated from a number of species of the jellyfish Aequorea. It is a 22 kilodalton [kD] molecular weight peptide complex [see, e.g., Shimomura et al. (1962) *J. Cellular and Comp. Physiol.* 59:233–238; Shimo mura et al. (1969) *Biochemistry* 8:3991–3997; Kohama et al. (1971) *Biochemistry* 10:4149–4152; and Shimomura et al. (1972) *Biochemistry* 11:1602–1608].

The aequorin photoprotein, which includes bound luciferin and bound oxygen that is released by Ca^{2+}, does not require dissolved oxygen. Luminescence is triggered by calcium, which releases oxygen and the coelentrazine substrate producing apoaqueorin. The aequorin system is well known [see, e.g., Tsuji et al. (1986) "Site-specific mutagenesis of the calcium-binding photoprotein aequorin, " *Proc. Natl. Acad. Sci. USA* 83:8107–8111; Prasher et al. (1985) "Cloning and Expression of the cDNA Coding for Aequorin, a Bioluminescent

Calcium-Binding Protein," *Biochemical and Biophysical Research Communications* 126:1259–1268; Prasher et al. (1986) *Methods in Enzymology* 133:288–297; Prasher, et al. (1987) "Sequence Comparisons of cDNAs Encoding for

Aequorin Isotypes," *Biochemistry* 26:1326–1332; Charbonneau et al. (1985) "Amino Acid Sequence of the Calcium-Dependent Photoprotein Aequorin," *Biochemistry* 24:6762–6771; Shimomura et al. (1981) "Resistivity to denaturation of the apoprotein of aequorin and reconstitution of the luminescent photoprotein from the partially denatured apoprotein," *Biochem. J.* 199:825–828; Inouye et al. (1989) *J. Biochem.* 105:473–477; Inouye et al. (1986) "Expression of Apoaequorin Complementary DNA in *Escherichia coli*," *Biochemistry* 25:8425–8429; Inouye et al. (1985) "Cloning and sequence analysis of cDNA for the luminescent protein aequorin," *Proc. Natl. Acad. Sci. USA* 82:3154–3158; Prendergast, et al. (1978) "Chemical and Physical Properties of Aequorin and the Green Fluorescent Protein Isolated from *Aequorea forskalea*" *J. Am. Chem. Soc.* 17:3448–3453; European Patent Application 0 540 063 A1; European Patent Application 0 226 979 A2, European Patent Application 0 245 093 A1 and European Patent Specification 0 245 093 B1; U.S. Pat. No. 5,093,240; U.S. Pat. No. 5,360,728; U.S. Pat. No. 5,139,937; U.S. Pat. No. 5,422,266; U.S. Pat. No. 5,023,181; U.S. Pat. No. 5,162,227; and SEQ ID Nos. 5–13, which set forth DNA encoding the apoprotein; and a form, described in U.S. Pat. No. 5,162,227, European Patent Application 0 540 063 A1 and Sealite Sciences Technical Report No. 3 (1994), is commercially available from Sealite, Sciences, Bogart, GA as AQUALITE®].

The native protein contains oxygen and a heterocyclic compound coelenterazine, a luciferin, [see, below] noncovalently bound thereto. The protein contains three calcium binding sites. Upon addition of trace amounts Ca^{2+} [or other suitable metal ion, such as strontium] to the photoprotein, it undergoes a conformational change the catalyzes the oxidation of the bound coelenterazine using the protein-bound oxygen. Energy from this oxidation is released as a flash of blue light, centered at 469 nm. Concentrations of calcium ions as low as 10^{-6} M are sufficient to trigger the oxidation reaction. Thus, exposure to Ca^{+2} triggers the light reaction, so that production of light is a measure of Ca^{+2} concentration. If the rate of the reaction is controlled, the aqueorin system is an ideal Ca^{+2} sensor.

This problem has been solved by encapsulating the Aqueorin protein in a sol gel to produce an optical sensor [Aylott et al. (1996) Abst. presented at the 3rd European Conference Optical Chemical Sensors and Biosensors, Zurich, Switzerland]. The photoprotein Aequorin was encapsulated into sol gel matrix to provide to slow the interaction between the Aqueorin photoprotein and the Ca^{+2} in the sample. This permits highly sensitive measurements of Ca^{+2} concentration. As modified herein, the a photodetector and memory will be incorporated into the gel, thereby permitting the light events to be detected and monitored.

For implementation using the memories provided herein, the power, logic and control electronics are encased within a protective, chemical-impervious, light transmissive shell, then submerged in the still-liquid sol, so that the shell is coated with the matrix material. Since the reaction produces its own light, a separate light source is not required, and only a photodetector need be included in the device. The photodetector is selected to detect light at the wavelength emitted by the photoprotein. At this point, the photoprotein, in this case, aequorin, has already been added to the sol.

The resulting sensor may be implantable, or may be placed as a separate unit into a solution, or built into a solution-containing vessel, such as a blood-drawing test tube.

Other sol-gel sensors with memories

Other types of photosensors similarly may be encapsulated in a sol-gel shell coating the electronic "capsule" for providing sensors for detecting other chemicals. The chemicals which may be detected include dissolved oxygen (O_2) and carbon monoxide (CO_2), using hemoglobin (Hb) or myoglobin (Mb) encapsulated in the sol-gel, both of which reversibly bind O2 to generate direct optical emission at 436 nm. The response time of the reaction is about one minute, and the sensor is stable for a few days. Dissolved nitric oxide (NO) can be optically detected using manganese myoglobin (MnMb). The presence of NO causes a sol-gel matrix containing the manganese myoglobin to emit light at 436 nm. This sensor is also stable for a few days. Dye-mediated detection of glucose and oxalate can be performed using the glucose oxidase and oxalate oxidase, respectively, with an enzyme co-factor (NADH), also encapsulated within the sol-gel. A glucose detector so constructed emits light at 510 nm and the oxalate detector emits light at 590 nm. Both of these sensors are stable over several months.

EXAMPLE 12

Blood Donor Bag

In order to assure that all donated blood is properly identified and tested, blood bank personnel must complete a significant amount of paperwork and must perform careful checks and double-checks of the donor's information and correct labeling of the bag(s) and vials containing the blood. Once properly identified, the vials are forwarded to a laboratory where it is separated and tested for a number of viral diseases. This procedure could be greatly simplified, and perhaps even automated, except for the nurse who draws the blood, by affixing the above-described sensors within the bags to record donor information, perform various tests on the blood, and automatically record the test results for later reading.

An exemplary embodiment of the "smart" blood bag is provided in FIG. **64**, with the sensor array **644** located at one side **646** of bag **642**. A single microprocessor **648** and memory **6410** are shown as this facilitates a one step remote write and read step, however, a dedicated microprocessor and memory can be provided for each individual sensor, each combination having a unique address to permit distinction between the different combinations during write and read steps. The microprocessor **648**, memory **6410**, battery **6412** and transceiver components **6414** are configured much like the corresponding components of Example **7**, with connections provided between the microprocessor **648** and each of the individual sensors. The temperature sensor of Example **7**, or a similar sensor **6416**, is also included, to record exposure of the sample to excessive heat, which might render the blood unusable and/or could lead to incorrect test results, and to provide temperature compensation for those tests that are temperature sensitive.

The infectious diseases for which the donor blood is typically tested include hepatitis B and C, HIV/AIDS, and human T-cell lymphotropic virus (HTLV). A sensor will be provided for each test performed, and multiple tests may be performed for a single disease in some cases.

As is known, the test for identification of the viral diseases of concern are conducted by immunoassay, i.e., measurement of the bioactivity (specific binding) between an antigen and an antibody. For hepatitis B, the tests may be for Hepatitis B surface antigen (Anti-HBs), or Hepatitis B core antibody (Anti-HBc); for Hepatitis C, the test is for Hepatitis C virus antibody (Anti-HCV); for HIV/AIDS, the different tests are HIV-1/HIV-2 antibody, HIV-1 (Western blot), and HIV-1 p24 antigen; and for HTLV, the HTLV-1 antibody.

Most bioanalytic sensors are based upon the redox immobilization of enzymes. These proteins catalyze the conversion of the analyte between two redox states that can subsequently be detected at the electrode. Redox enzymes include two large groups: the oxidase enzymes and the dehydrogenase enzymes. Both types of enzyme catalysis can be mediated by a variety of species that act as electron acceptors. The most common mediators used in biosensing techniques are ferrocene compounds. Such mediators can be incorporated into electropolymerized films.

For each of the above tests, a three electrode arrangement as in Example 7 is provided, i.e., a working electrode, a reference electrode and a counter electrode. Also as in Example 7, a membrane is placed over a portion of the electrodes to permit the electrical detection of immune reactants. This membrane may be as described for Example 7, or may be one of the electroactive polymers described below. The specific antigen is conjugated with the enzyme, and both are retained within the membrane over the electrodes. Enzymes that may be used include, for example, horseradish peroxidase, alkaline phosphatase, glucose oxidase and β-galactosidase. An appropriate ferrocene compound incorporated into a thin film can be placed between the buffer layer over the electrodes and the enzyme/antigen membrane.

The microprocessor is programmed to scan the voltage applied to the working electrode between a maximum and a minimum level at a fixed scan rate. The scan rate varies from a few millivolts (mV) per second to a few hundred millivolts per second. This procedure is commonly used in biosensors, and is known as cyclic voltammetry [see, e.g., Foulds, et al. "Immunoelectrodes", in *Biosensors—A Practical Approach*, edited by A. E. G. Cass, IRL Press, 1990]. The current is then measured at the working electrode. This current has two components: a capacitive or non-Faradic component arising from redistribution of charged and polar species at the electrode surface, and a Faradaic component resulting from exchange of electrons between the electrode and redox active species at the electrode surface. If the potential is sufficiently oxidizing or reducing and the rate of electron transfer between the electrode and the redox species in solution is sufficiently fast, the Faradaic current is controlled by the rate of diffusion of the electroactive species to the electrode.

As the voltage is swept upwards (more positive) the oxygen concentration drops, indicated by an increase in current. The current reaches a maximum value, then decays. Upon reversal of the voltage, the current will again increase as a result of re-oxidation. The Faradaic component of the current must be extracted from the combined current. The non-Faradaic component of the current can be determined by the tangent to the initial slope during the first sweep. These procedures can be programmed into the microprocessor, so that a final value can be stored in memory, or the raw data can be stored for later processing once it is retrieved by the remote reader.

Referring again to FIG. **64**, once the bag is filled with blood, small capillary tubes **6418** distribute the blood to the different test sites. The capillary tubes are configured for one-way flow so that the small amounts of blood separated out cannot leak back into the main part of the bag. After waiting the appropriate time for the tests being conducted, the user scans the bag to verify donor identity and to obtain the stored test results.

As is known, other types of testing may be used to produce electrical signals which can generate data for storage indicative of the test results of each of the immunoassays. Alternative sensors types include thermometric sensors [which may include the use of sequentially acting enzyme for amplification of the total enthalpy change], and optical sensors, e.g., a Western Blot test, as further described below.

The Western Blot test uses a film strip upon which the proteins, such as those from human immunodeficiency virus [HIV] are laid out vertically in order of molecular size, from largest to smallest, on a strip of film or paper. Such a strip **652** is embedded in the test area of the blood bag with open space over the strip for exposing the strip to the blood, as illustrated in FIG. **65**. The appropriate enzyme is contained within a release control system which is activated by a signal generated by the device's microprocessor within the device's electronics **654**. The electroresponsive hydrogel described in U.S. Pat. No. 5,152,758 to Kaetsu, et al. is one such control system. The electroactive polymers described by J. N. Barisci, et al in "Conducting Polymer Sensors", *TRIP*, Vol.4, No. 9, September 1996, pp. 307–311, may be utilized in a similar manner. These materials are polymers into which physiologically active substances, including enzymes, can be incorporated during or after copolymerization, and which will release the physiologically active substance upon application of a voltage. In this case, the enzyme-containing polymer **656** is coated onto an electrode **658** which is positioned adjacent the film **652**. Release of the enzyme is timed by the microprocessor to occur after a sufficient delay to permit binding of any HIV antibodies and with the antigens. A second electrode **6510** coated with an electroactive polymer **6512** containing appropriate chemical dye is used to release the chemical dye for marking the bound antibodies and antigens.

The strip film **652** can have a reflective backing or can be transparent or opaque. For a reflective backing, a photodetector **6514** positioned over the strip will measure the amount of light reflected. For a transparent strip or opaque, the photodetector is positioned behind the strip. A second, reference photodetector **6516** is provided to provide a baseline measurement of the light intensity, which can be ambient light, light from an external light source, or light from an LED included in the sensor device. The areas of the strip that are darkened **6520** by the chemical dye will absorb more light, reducing the total reflected intensity. An electrical signal representative of the detected level of light is then written into the device's memory for comparison to a threshold indicative of HIV infected blood, the threshold being determined statistically based upon the minimum surface area of the strip that will be darkened in a positive test.

EXAMPLE 13

Glucose Sensor #2

The power, logic and communication electronics of Example 7 are again used in this embodiment of a glucose sensor, which is illustrated in FIG. **66**. The electrodes **664,666,668**, however, are formed on the surface of the shell **662** which surround the electronics, providing a more compact, implantable sensor. The shell is coated with an electroactive polymer such a polypyrrole, a polythiophene, or a polyaniline [see, Barisci et al. (1996) "Conducting Polymer Sensors", *TRIP* 4:307–311 for a description of such polymers]. The use of electroactive polymers permits the direct incorporation of the catalyzing enzyme into the polymer, rather than requiring the physical formation of a well or cavity in which an enzyme-containing membrane is to be retained.

For a glucose sensor, a polypyrrole (PPy) coating is formed on the surface of shell **662**, through which conduc-

tive connectors **6610** extend for connection to the electrodes. The electrodes **664,666,668** are defined by selective laser ablation of the coating to remove the material between the electrodes. Alternatively, photolithographic techniques may be used. The GOx is then adsorbed into the electrodes. It may also be desirable to incorporate a ferrocene compound as a mediator into the electrodes. Operation of the PPy electrode-based sensor will be similar to the procedure for the sensor described in Example 7.

Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

What is claimed:

1. A library of molecules or biological particles comprising:

a plurality of unique molecules or biological particles, wherein each molecule or biological particle is linked to and synthesized on one of a plurality of discrete solid supports, wherein each solid support comprises a combination of:

a matrix comprising a material treated for linking molecules or biological particles and an information surface for imprinting or engraving; and

an optically-readable symbol imprinted or engraved on the information surface, the symbol comprising optically-encoded data including a unique identifier and an orientation indicator for determining orientation of the matrix with respect to an optical reader;

wherein the unique identifier corresponds to the molecule or biological particle that is linked to and synthesized on the material for linking so that each molecule or biological particle of the library is uniquely labeled.

2. The library of claim **1**, wherein the matrix comprises a porous non-collapsible vessel formed from an inert material and the material for linking comprises a plurality of particles disposed within the vessel.

3. The library of claim **2**, wherein the particles are derivatized for linking the molecules or biological particles.

4. The library of claim **2**, wherein the vessel comprises a frame formed from an inert material which supports an inert mesh and a cap inserted into the frame.

5. The library of claim **4**, wherein an exterior surface of the cap comprises the information surface.

6. The library of claim **2**, wherein the vessel has a volume of about 30 mm^3 or less.

7. The library of claim **1**, wherein the information surface is formed from a material that is different from the material for linking.

8. The library of claim **1**, wherein the information surface is formed from a material selected from the group consisting of polypropylene, glass, polytetrafluoroethylene, polyethylene, polystyrene, polyester, ceramic or composites thereof.

9. The library of claim **1**, wherein the material for linking is a radiation-grafted polymer that is derivatized for linking the molecules or biological particles.

10. The library of claim **1**, wherein the optically-readable symbol is a two-dimensional bar code.

11. The library of claim **1**, wherein the optically-readable symbol comprises a plurality of holes or pits formed in the information surface.

12. The library of claim **1**, wherein the molecules or biological particles are selected from the group consisting of monomers, nucleotides, amino acids, small molecules, antigens, antibodies, ligands, proteins, nucleic acids, phage and viral particles, and cells.

13. The library of claim **1** that is a combinatorial library.

14. A library of molecules or biological particles comprising:

a plurality of unique molecules or biological particles, wherein each molecule or biological particle is linked to and synthesized on one of a plurality of discrete solid support devices, each solid support device comprising the combination of:

a vessel comprising a frame formed from an inert material and an inert mesh supported by the frame, the mesh adapted to permit reagents to pass therethrough; wherein the vessel has an information surface;

a plurality of particles contained within the vessel, wherein the particles are treated for linking molecules or biological particles; and

an optically-readable symbol imprinted or engraved on the information surface, the symbol comprising optically-encoded data including a unique identifier and an orientation indicator for determining orientation of the symbol with respect to an optical reader;

wherein the unique identifier corresponds to the molecule or biological particle that is linked to and synthesized on the particles contained within the vessel so that each molecule or biological particle of the library is uniquely labeled.

15. The library of claim **14**, wherein the frame is adapted for receiving a cap for sealing the vessel.

16. The library of claim **15**, wherein an outer surface of the cap comprises the information surface.

17. The library of claim **14**, wherein the vessel has a volume of about 30 mm^3 or less.

18. The library of claim **14**, wherein the optically-readable symbol is a two-dimensional bar code.

19. The library of claim **14**, wherein the molecules or biological particles are selected from the group consisting of monomers, nucleotides, amino acids, small molecules, antigens, antibodies, ligands, proteins, nucleic acids, phage and viral particles, and cells.

20. The library of claim **14** that is a combinatorial library.

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